Quantifying the Effectiveness of Wetland Restoration in a Tidally Dominated System

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
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ABSTRACT

Coastal wetlands provide valuable ecosystem services such as water quality improvement, carbon burial, and habitat creation. However, there has been a significant decline in wetland area as development and sea level rise threaten coastal habitats. Coastal wetland creation and restoration projects have been implemented to preserve these ecosystem services. We evaluated the effectiveness of the wetland restoration methods used at the North Carolina Coastal Federation's North River Wetland Preserve (NRWP), Carteret County, NC. The NRWP converted 6,000 acres of farmland into wetlands beginning in 1999. We used a chronosequence of three wetland restoration sites at the NRWP to evaluate how habitat quality (expressed as invertebrate abundance and diversity), denitrification rates, and carbon burial change over time. Additionally, we assessed water quality changes by comparing loads of nutrients, pathogenic bacteria, and fecal indicator bacteria from the restored wetland versus adjacent farmland, the wetland's previous state. Significant differences in hydroperiods among sites confounded our ability to assess change over time and likely drove variation in habitat quality, carbon burial, and vegetation cover. Marsh thickness is correlated with age. Similarly-high denitrification rates (~700 $\mu M/m^2/hr$) were found across restored and natural wetland sites. Higher concentrations and loads of Vibrio spp. and fecal indicator bacteria were observed in farmland outfalls than wetland outfalls. Results presented here provide insight for future methods of restoration that optimize the provision of ecosystem services.

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INTRODUCTION

Since 1982, the North Carolina Coastal Federation (NCCF) has worked to improve water quality and ecosystem services in NC's coastal communities (NCCF, 2020). Some examples of their work include maintaining and restoring water quality to meet recreational standards, promoting habitat creation and reducing coastal erosion with living shorelines, and restoring collapsed oyster populations in NC coastal waters (NCCF, 2020). In 1999, NCCF acquired North River Farms, 6,000 acres of row-crop farmland located downstream of the 45,000-acre Open Grounds farm (NRWP, 2020). Agricultural runoff from a large portion of Open Grounds, which primarily grows corn and soybeans, flows through North River Farms on its way to the North River (Open Grounds, 2020). Since that time approximately 4,200 acres of North River Farms, renamed the North River Wetlands Preserve, have been either restored to or preserved in a natural state; the remaining 1,800 acres are in the process of or planning to be restored to a wetland (NRWP, 2020).

Agricultural runoff often contains a suite of water-quality contaminants, such as elevated nutrient levels, pesticides, pathogens, sediment, salts, and trace metals (O'Geen et al., 2010). To combat these threats to water quality, restored wetlands can be constructed with the potential to remove or retain many water-quality contaminants in agricultural runoff. However, to be successful, restored wetlands must be carefully designed and managed (O'Geen et al., 2010). NCCF hoped to meet this potential through the creation of North River Wetlands Preserve. The specific goals of the NCCF for this wetland restoration project were:

1. Reduce agricultural runoff and improve water quality
2. Eliminate the surge of contamination after rain events and prevent shellfish closures
3. Restore oyster habitat and shell fishing
4. Create a range of habitats for fish, birds, and other wildlife
5. Promote research, education, and public involvement

This study aimed to quantify the extent to which the NCCF met these goals as well as the overall success of the restoration project, as of the growing season from August to November 2020. We employed a variety of analyses to assess the effectiveness of restoration. In order to address each of the goals of the NCCF we assigned teams to study hydrology, carbon burial, water quality, and habitat quality. These topics were chosen to provide the most well-rounded view of how the provisioning of ecosystem services has improved since the restoration. The restoration project evaluated in this study incorporated three wetland restoration treatments within the North River Wetlands Preserve constructed in 2007, 2013, and 2015. To evaluate the water quality before and after entering the restoration site, this study looked at agricultural runoff from two farm outflows and released water from two wetland outflows (Fig 0.1). The main research questions each team asked were:

1. How do the ecosystem benefits of the restored wetland compare to its previous state (row crop agriculture)?
2. How do ecosystem functions in the restored wetland change over time and how do they compare to natural wetlands?

From these questions each team designed a set of experiments focused on their specific topic. We hope the results of these studies will inform the future restoration practices at the North River Wetland Preserve.

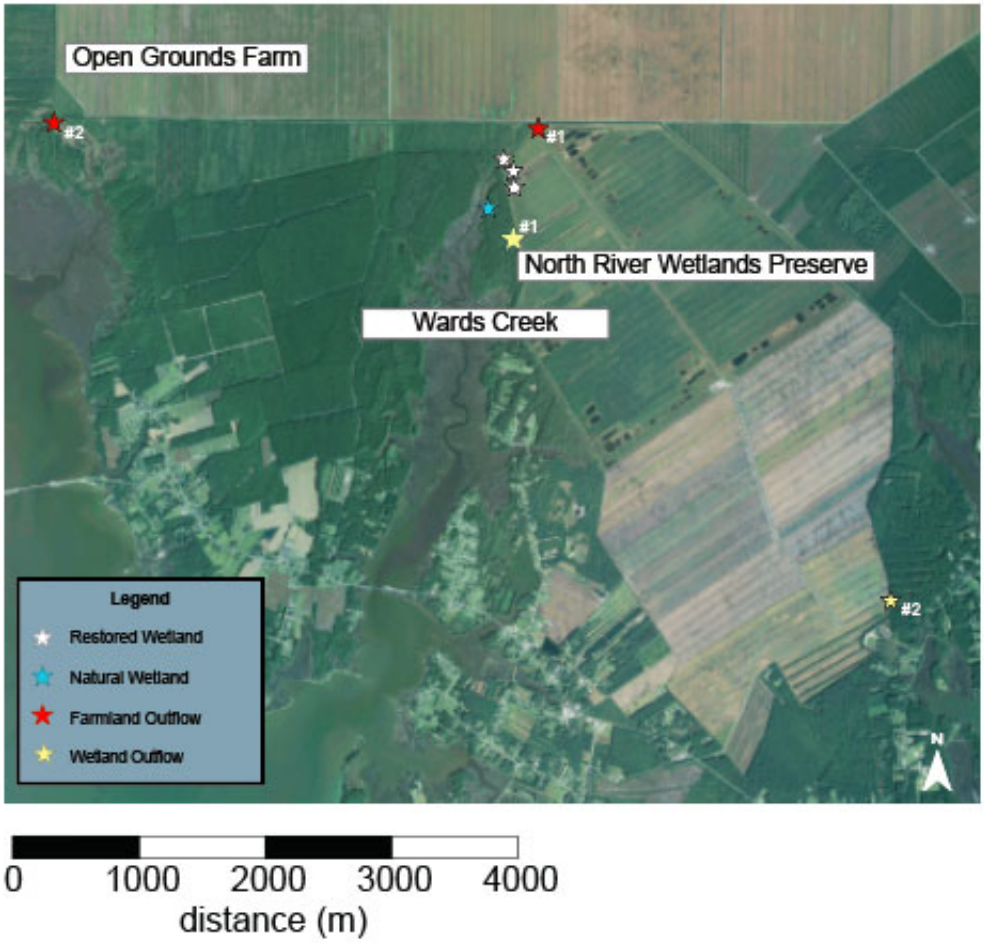

Figure 0.1 Map of the three restored wetlands and the natural wetland, as well as the four outfalls used in this study.

CHAPTER 1: Hydrology

1. INTRODUCTION

A coastal wetland can be defined as a terrain that is subject to wetting by coastal processes, including influences from daily tides, storm surge, and wave swash (Semeniuk & Semeniuk, 2016). This characteristic inundation of coastal wetlands functions is critical for a suite of valuable ecosystem services such as nutrient and pollution uptake, particle deposition, water flow regulation, wave and wind attenuation, and habitat creation (Barbier, 2013). However, globally, there have been significant losses in coastal wetlands as development and sea-level rise threatens coastal habitats (Duarte, 2009).

Coastal wetland creation and restoration projects have been implemented in an effort to preserve these ecosystem services, though environmental threats such as sea-level rise still pose risks to these systems. Studies have shown that a created marsh that displays elevation dynamics similar to that of a natural marsh and is constructed with a high elevation capital can be resilient to current local rates of relative sea-level rise (Kurki-Fox et al., 2019; Jarzemsky et al., 2013). However, there is a need for further observations to evaluate the system dynamics that provide this resilience to support wetland research and management objectives (Kurki-Fox et al., 2019).

Due to their potential to improve water quality, restored wetlands have gained attention as an agricultural runoff mitigation strategy (Vymazal & Březinová, 2015). Engineering a site to the appropriate hydroperiod is critical to retaining agricultural runoff and the overall success of wetland restoration (Jarzemsky et al., 2013). An insufficiently-high water table results in an increased chance of subsidence of stored carbon and the occurrence of peat fires. In the context of habitat quality and creation, water level dictates the zonation of vegetation and community structure (Kurki-Fox et al., 2019). Hydrologic data are essential for characterizing the relationships between wetland morphology and function, as well as understanding the driving forces behind ecosystem services.

Hydroperiod refers collectively to the timing, frequency, and duration of inundation of a wetland (Hamilton, 2009). Subtle differences in topography can result in considerable differences in hydroperiod with corresponding variation in ecological characteristics (Hamilton, 2009). Thus, successful wetland restoration and creation are dependent on the ability to recreate the hydrologic regimes of functional wetlands (Shaffer et al., 1999). Succession and ecosystem development of most marshes is driven by allogeneic factors such as hydroperiod, salinity, and disturbance, all of which maintain the herbaceous vegetation against encroachment by woody vegetation (Craft, 2016). Hydroperiod is the primary driver of marsh structure, particularly in regions with unstable climates (Craft, 2016).

In this short-term study, we evaluated the hydroperiod of three wetland restoration projects constructed in 2007, 2013, and 2015 within the North River Wetlands Preserve in Carteret County, North Carolina. A natural wetland located in the same estuarine system and with close proximity to the restored wetlands was also evaluated. The three restored wetlands have been converted from farmland and consist of highly variable elevation patterns and vegetation compositions. We monitored the inundation frequency and aerial extent of inundation by combining topographical data with water level data, and hypothesized that the hydroperiod of the restored wetlands would vary by wetland design and would be dissimilar from a natural wetland. With this information, we have evaluated the success of the three restored wetlands in the context of hydrology and provide insight into the quality of the wetland design. The specific

objectives of this study included: 1) Evaluate how the hydroperiod varied within and among restoration treatments and 2) Quantify how closely the hydrology of the restored wetlands reflected a natural salt marsh.

2. METHODS

2.1 Aerial Imagery

Aerial imagery of all wetland sites was taken using a DJI Mavic Pro drone. An orthomosaic was then created using Pix4D.

2.2 Elevation Data

Topographical data of the 2007, 2013, 2015, and natural wetland was collected using an R8s RTK Trimble backpack GPS receiver. Transects were taken in cross-hatched directions with a maximum precision of 8 mm Horizontal / 15 mm Vertical. Points were recorded every 1.0 m along the transects, with between 300-1300 points taken in each marsh (Table 1). The aerial imagery and topographical data were imported into Surfer® (Golden Software, LLC) to generate a map of the elevation of any given point within the site using the kriging algorithm with a resolution of approximately 1 m^2 (Table 1).

Table 1
[a] The number of elevation-data points collected in each wetland site
[b] The number of grid-blocks interpolated

Wetland	Elevation Measurements [a]	Grid-Blocks [b]
2007	1267	11186
2013	1154	11105
2015	1247	7369
Natural	384	4743

2.3 Water Level Data

Two HOBO U20-001-04 Water Level Data Loggers were deployed in the channel (Fig. 1A). One logger was located near the wetland restored in 2007, and the other was located next to one of the farm's outflow pipes (Table 2). A HOBO U20L-04 Water Level Data Logger was installed in a tree bordering the channel to account for changes in atmospheric pressure (Table 2).

Table 2. Location and deployment period of all instruments deployed in this study.

Description	Abbreviation	Latitude	Longitude	Deployment Period
Water level logger in the channel	WL1	34.81873	-76.56026	8/27/20 – 10/24/20
Water level logger at the farm outflow pipe	WL2	34.82143	-76.55742	9/8/20 – 10/14/20
Atmospheric pressure logger located in a tree between WL1 and WL2	BL	---	---	8/27/20 – 10/14/20

WL1, WL2, and WL3 were installed inside PVC stilling wells, according to the Onset Tech Note on constructing a stilling well (Onset, 2020). The elevation of WL1, WL2, and WL3 was taken using an R8s RTK Trimble to convert water level recordings to the North America Vertical Datum of 1988 (NAVD88). The loggers recorded water level every 5 minutes with a typical error of ±0.075% (±0.3 cm) and remained deployed for an entire lunar cycle. Data from all loggers were retrieved on a biweekly schedule.

2.4 Percent Inundation

Data from WL1 is assumed to be representative of the water level across all wetland sites under consideration. We used the strong correlation between WL1 and WL2 data along with the relatively close proximity of all wetlands as validation of this assumption.

To determine the inundation frequency across each marsh, we first created an array of 100 evenly spaced elevation bins from the minimum observed water level to the maximum observed water level. The time series of WL1 water level data (NAVD88; m) was then used to determine the percent occurrence of each elevation bin in the array.

By assuming that any elevation below the minimum observed water elevation is inundated 100% of the time, we determined the percent inundation (in percent of time) of each subsequent elevation bin according to Equation 1:

$$\%Inundation_{n+1} = \%Inundation_n - \%Occurrence_{n+1} \quad (1)$$

where *n* refers to the position in the array of elevation bins.

Through this process, we generated a transform curve detailing the percent inundation of each elevation value. Using our transform curve, percent inundation was interpolated for every 1 m^2 grid-block elevation value for each wetland in MATLAB R2020a (The Mathworks, 2020).

3. RESULTS

3.1 Wetland Design

Aerial imagery revealed distinct structural choices made in the design and construction of each of the three restored wetlands as well as the existing geomorphology of the natural wetland. The natural wetland is located off Wards Creek (Fig. 1A), and the four restored wetlands are connected to this creek through excavated channels and internal drainage structures. The 2007 wetland has a narrow meandering channel feature that begins in the southwest corner and runs along the northern edge of the wetland. The 2013 wetland was built with a series of irregularly sized and connected pond-like depressions which are located throughout the site. The 2015 wetland has a dendritic channel feature which begins with a wide mouth in the northwest corner and stretches towards the center of the site with narrow branches reaching in the direction of the other three corners of the wetland. The three restored sites are connected to Wards Creek through a wide excavated channel which runs along the long edge of the 2007 site as well as two smaller channels which run along the western edges of each site. The natural wetland has a channel which branches off of Wards Creek towards the eastern edge of the site. This channel formed naturally, unlike the engineered channels in the restoration sites (Fig. 1A).

Elevation data overlaid on the aerial imagery as a color relief map further elucidates geomorphological variation within and across the wetland sites. Each of the restored wetland sites has a relatively constant elevation profile outside of their drainage structures. The 2007 and 2015 wetland see most grid blocks falling between approximately 0.35 m and 0.65 m elevation, with mean elevations of 0.35 m and 0.36 m respectively. The 2013 wetland sees most grid blocks falling between approximately -0.05 m and 0.15 m elevation with a mean elevation of 0.056 m. The natural wetland site saw an elevation profile which consisted of clear depressions in the center and elevated regions along the edges of the site. The mean elevation of the natural wetland was 0.11 m.

The hydrological features of the sites also see variation in elevation. The 2007 wetland channel is generally between -0.25 m and -0.10 m elevation at its mouth, but its elevation increases rapidly towards the northern edge of the site. The central portion of the 2015 channel from the mouth is approximately -0.40 m to -0.30 m elevation, but the branches are much shallower at about -0.05 m to -0.05 m. The 2013 wetland sees some of the lowest elevations in its pond features with points as low as -0.50 m (western edge), though most of the depressions are at approximately -0.30 m to -0.15 m elevation. This elevation pattern mimics that of the depressions in the natural wetland site. The 2007 and 2015 wetland sites tended to be of similar and higher elevation and 2013 and the natural wetlands were of similar and lower elevation on average (Fig. 1B, Table 2).

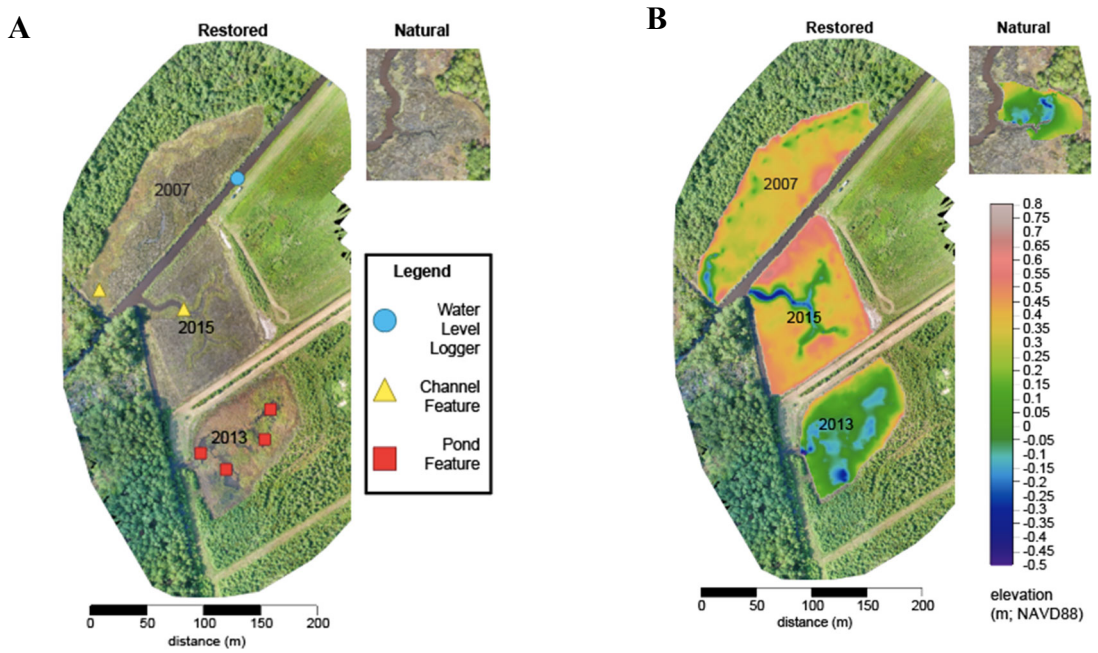

Figure 1. Maps displaying excavated features within the restoration sites (A) and constructed and natural morphology of both the restoration and natural sites (A, B). Elevation (m, NAVD88) is displayed as color relief and has a resolution of approximately $1m^2$ (B).

Table 3. Summary statistics generated from elevation (m; NAVD88) collected in the field.

Site	Minimum	Maximum	Mean	Median	Variance	St. Dev.
2007	-0.279	0.704	0.346	0.356	0.013	0.113
2013	-0.656	0.691	0.056	0.020	0.028	0.169
2015	-0.486	0.798	0.363	0.406	0.033	0.181
Natural	-0.459	0.563	0.111	0.118	0.027	0.165

3.2 Water Level and Inundation

The time series of water data (Fig. 2A) revealed a tidal range of 0.63 m, a microtidal environment. WL1, used to create the transform curve (Fig. 3), had a mean water level of 0.18 and WL2 had a mean water level of 0.26 m. We attributed this difference to bottom friction maintaining a surface level gradient along the channel. We based this conclusion upon a reasonable value for the coefficient of friction found when flow values and the distance between the two water level loggers was considered.

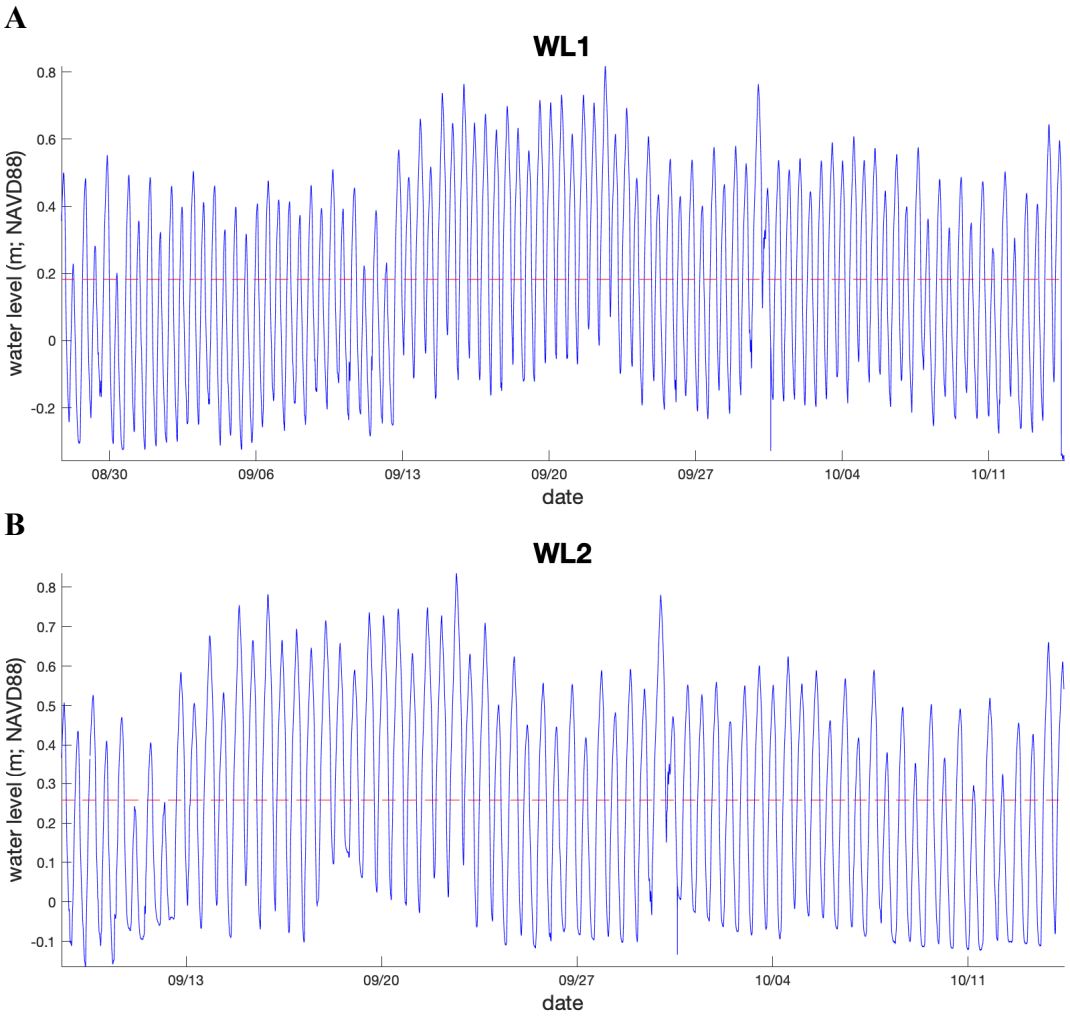

Figure 2. Time series of water level data from WL1 (A) and WL2 (B). The mean water level is indicated by the red dashed line.

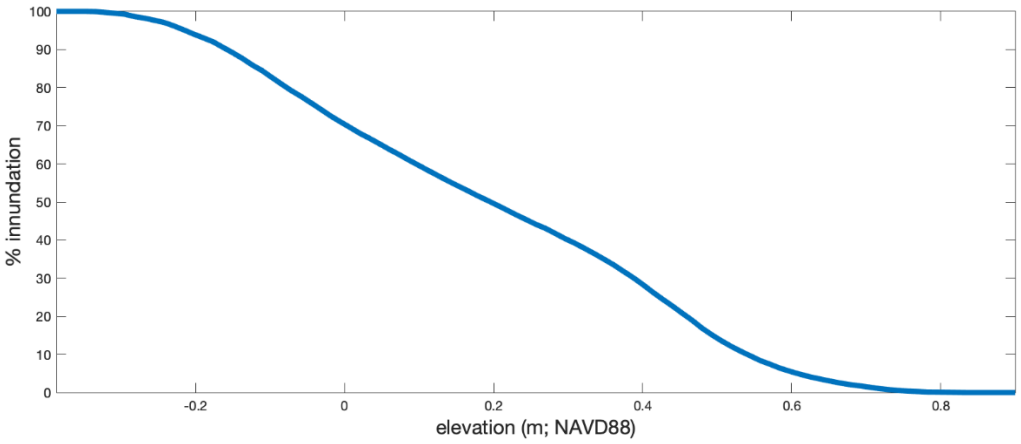

Figure 3. Transform-curve showing the relationship between an elevation and its corresponding percent inundation value. This curve was used to interpolate the percent inundation of each wetland (per unit area).

Despite differences in restoration design and elevation among sites, every grid block of the restoration sites was inundated for some amount of time during the monitoring period. The interpolated average percent inundation was highest for the 2013 wetland, followed by the natural, 2007, and 2015 wetland sites (Fig. 4, Table 4).

Percent inundation over the monitoring period was graphed against percent area for each of the four sites (Fig. 5). Approximately 50% of the 2007 wetland was inundated between 35 and 40% of the monitoring period with a peak at 40% and a mean at 35.4% inundation. Approximately 50% of the 2013 wetland was inundated between 75 and 95% of the monitoring period with a peak at 80% and a mean at 65.3%. Approximately 50% of the 2015 wetland was inundated between 20 and 35% of the monitoring period with a peak at 30% and a mean at 31.6%. The natural wetland followed more of a bimodal distribution with peaks at 40-45 and 80% inundation and a mean at 59.4%. The 2007 and 2015 wetlands had strong peaks with right skews. The 2013 and natural wetlands had weak peaks and left skews, seeing a more even spread across inundation regimes from 30-90 percent inundation. The 2013 and natural wetlands generally saw greater inundation frequency over their area than the 2007 and 2015 wetlands (Fig. 5, Table 4).

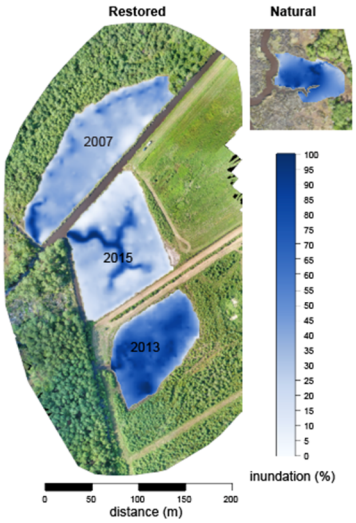

Figure 4. Color relief maps displaying elevation inundation (%) over the water level logger deployment period across all four sites with a resolution of approximately 1m^2.

Table 4. Summary statistics for the interpolated inundation frequency (%) data.

Site	Minimum	Maximum	Mean	Median	Variance	St. Dev.
2007	2.0	97.4	35.4	35.3	149.8	12.2
2013	4.1	100.0	65.3	68.0	324.5	18.0
2015	0.1	100.0	31.6	27.4	396.6	19.9
Natural	8.0	100.0	59.4	57.7	310.9	17.6

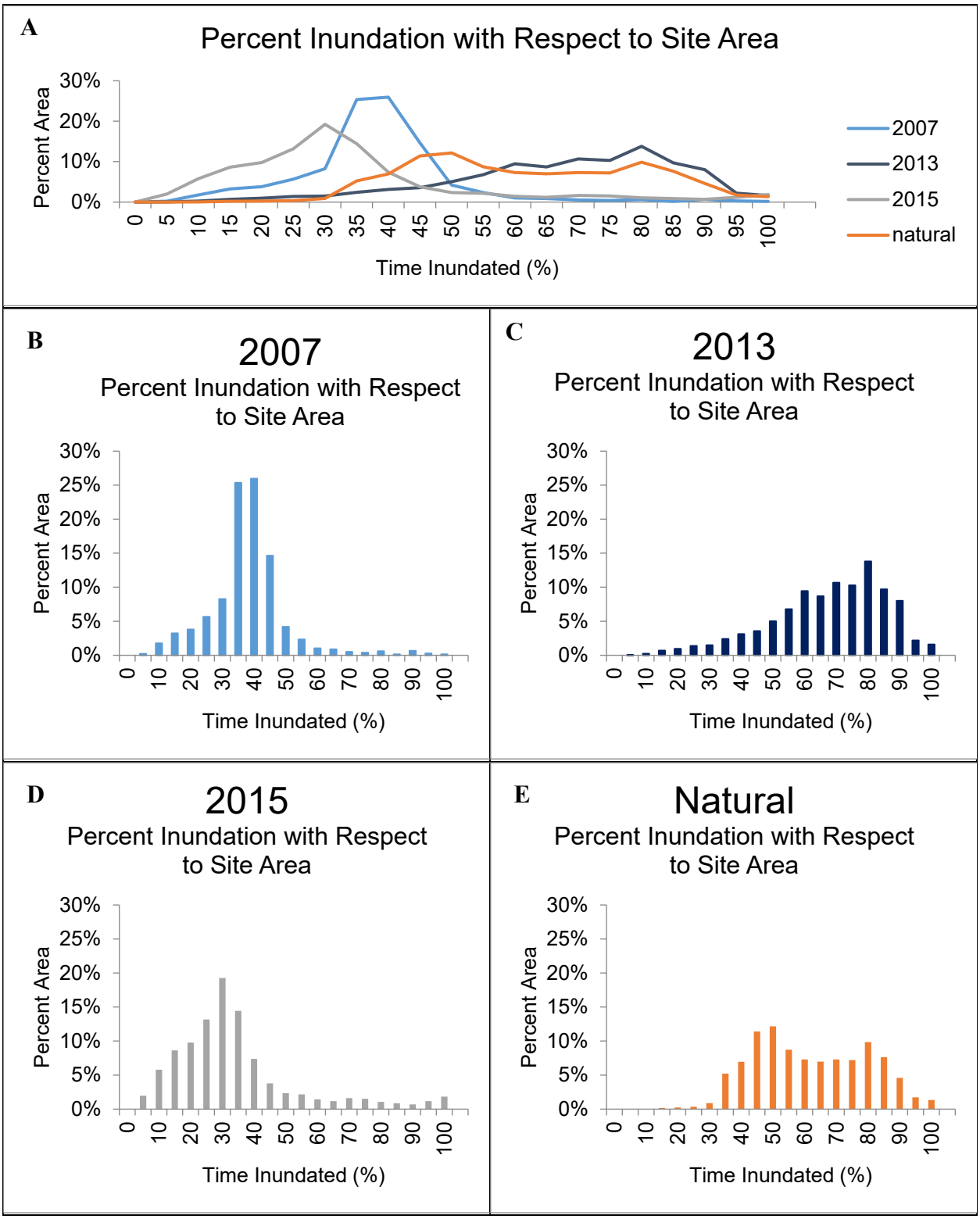

Figure 4. Histograms for each of the four sites (B-E) express the frequency at which average percent inundation values occur over the wetland area. These histograms were combined and simplified a line graphs (A) for visual comparison.

4. DISCUSSION

4.1 Inundation and Wetland Construction

Our maps and analysis show that all three restoration sites in the chronosequence were inundated to some degree during the deployment period of the water-level loggers, and that the hydroperiod of the 2013 wetland most closely resembled that of the natural wetland over the same period. The inclusion of ditches and lower elevations in the 2013 wetland restoration design likely promoted more frequent inundation, for longer durations, and to greater extents as compared to the 2007 and 2015 wetlands. The natural wetland had both ditch-like areas of lower elevation and a tidal channel (see Fig. 4, natural, lower left). The elevation of the tidal channel was not recorded due to connectivity issues with the RTK Trimble. We do not expect that including the tidal channel in our analysis would have altered our results significantly as it would have provided only a few extra grid blocks and these grid blocks would have been of sufficiently low elevation to maintain our conclusion that wetlands 2013 and natural were of higher inundation than 2015 and 2017. Our results suggest that the design of restoration sites with respect to elevation and connection to waterways can influence inundation in tidally-dominated regions, and that these should be considered in the construction process. Constructing a restoration site at elevations near the lower tidal limit of the local area with drainage structures (i.e. tidal fingers and ditches) which encourage inflow may result in greater inundation. This conclusion may be used to inform future restoration projects on designs that may enhance inundation frequency, intensity, and duration.

There are several ways in which saltmarsh wetland systems are affected by periods of inundation including increased rates of denitrification and nitrate retention (Shiau et al., 2016, Etheridfe et al., 2017), sediment accretion and carbon burial (Boerema et al., 2016.), and determination of habitat composition (Olff et al., 1988). In this study, we assume that any level of inundation provides some limited amount of ecosystem benefits, but understand that wetland success is likely also determined by other factors. We cannot say with confidence that the 2013 wetland site, for example, is any more effective in its generation of ecosystem services than the 2007 and 2015 sites solely on the basis that its hydrologic regime resembles that of the natural wetland. Other considerations discussed in the rest of this paper–habitat quality, denitrification rates, carbon burial, and nutrient and bacteria loading–will contribute to a comprehensive assessment of the effectiveness of restoration methods implemented at the NCCF North River Wetland Preserve.

4.2 A Tidally-Dominated Wetland

Because the restored wetland sites had strong tidal signals and other inputs were not accounted for through monitoring, we were unable to discern farm outflow and stormwater contributions from tidal influences on inundation frequency of the wetlands. Inundation of the wetland occurs with each rising tide as water enters the system through channels into wetland drainage structures. Monitoring inundation on a finer scale by measuring rainfall and outflow contributions might be informative in terms of understanding how the restored wetlands might mitigate the effects of these events. Also useful might be the inclusion of flow monitoring devices throughout the wetlands to understand the rate at which water moves throughout the system and further elucidate the effects of hydrology on wetland success.

Tidally-dominated wetlands see regular periods of inundation and drying as tides rise and fall. Though this connection to the sea provides a suite of ecosystem benefits, tidal marshes are

vulnerable to relative sea-level rise (RSLR) because they occupy a narrow elevation range. Loss of habitat and associated ecosystem services are of primary concern. Reconstructions of tidal marsh retreat and expansion during the Holocene indicated that marshes are nine times more likely to retreat than expand when RSLR rates are ≥7.1 mm/yr (Horton et al., 2018). Marsh elevation within the vegetation growth range (elevation capital) and the rates of marsh surface elevation change and RSLR are factors which can be used to predict the influence of sea-level rise on habitat loss. Marshes with low elevation capital are more likely to see flooding stress and deterioration, leading to an inability to support continuous coverage of salt marsh vegetation. Those with higher elevation capital and greater accretion rates may be better able to withstand the effects of RSLR (Cahoon et al., 2019). Migration of wetland habitat onto uplands is possible in some scenarios, but it is not a viable solution for marshes surrounded by steep uplands. High-elevation marshes may convert to low marsh, changing habitat types and ecosystem services. Systems with a limited suspended sediment supply may experience earlier and more rapid habitat conversion and marsh loss due to low accretion rates (Farron et al., 2020). However, some wetland services may be maintained despite sea-level rise. Tidal wetland carbon accumulation rate is projected to increase in this century and the wetlands studied are expected to continue their carbon sequestration capacity in all climate change scenarios modeled (Wang et al., 2019). In the face of sea-level rise, the restoration sites studied may see shifts in elevation, habitat type, and ecosystem function. Those which are currently at higher elevation may prove valuable in creating a more sustainable wetland system in the long-term. Long-term monitoring of sediment accretion rates, elevation, carbon sequestration, nutrient fluctuations, and habitat shifts has the potential to provide useful information about the success of different wetland designs in the context of RSLR.

5. CONCLUSION

This research evaluated how the hydroperiod varied within and among restoration sites and quantified how closely the hydrology of the restored wetlands reflected a natural salt marsh. We found that the structural design of the restoration sites influenced inundation patterns. The 2013 wetland most closely resembled the natural wetland with respect to elevation and percent inundation. This information might be used to inform future wetland restoration project design. Future research should evaluate the hydrology of the restored wetlands as it pertains to inputs from Open Grounds Farm and storm water runoff and flow. Wetland inundation may be an important driving factor for trends in habitat creation, carbon sequestration, and nutrient loading patterns. Long-term monitoring of these processes may reveal the effects of sea-level rise on wetland success.

CHAPTER 2: Carbon Sequestration

1. INTRODUCTION

Wetland ecosystems are highly efficient carbon sinks, sequestering carbon derived from living biomass and sediments transported onto the marsh platform during a tidal cycle (Doughty et al., 2015). Despite covering 4-6% of the terrestrial land surface, wetlands are estimated to account for one-third of the global soil organic carbon storage (Mitsch et al., 2012). There is increasing evidence that wetlands have an important and underestimated role in both carbon storage and the regulation of greenhouse gas emissions. As a result, wetlands are optimum natural environments for sequestering atmospheric carbon dioxide as carbonaceous sediment. Many studies have focused on quantifying the carbon held in terrestrial ecosystems which make up 95% of all wetlands in conterminous United States, but focus has shifted to the carbon held in tidal saline ecosystems (Nahlik & Fennessy, 2016).

Saltmarshes accumulate carbon through aboveground and belowground biomass as well as imported sediments on flood tides (McLeod et al., 2011). Marshes require sufficient sediment supply to maintain their position in the tidal frame. Wetlands receive carbon from both allogenic, from an external source on the flood tide, and autogenic, decaying plant matter, sources to accrete vertically. The hydrological connections between watercourses and their associated wetlands are important for the exchange of carbon and nutrients and are essential to the function and integrity of the wetland system. Waterlogging of wetland soils limits oxygen diffusion into sediment profiles creating anaerobic conditions. These conditions slow decomposition rates, leading to the buildup and storage of large amounts of organic carbon in wetland sediments (Foster et al., 2012). Wetlands are also involved in horizontal transport of carbon between ecosystems. They can trap carbon-rich sediments from catchments, but may also disperse carbon through water flow (Foster et al., 2012). These carbon contributions aid in vertical growth, which is necessary for wetlands to be resilient to sea level rise and continue to provide ecosystem services. Accelerating sea level rise and too little sediment transport can cause a marsh to drown or erode, thus reintroducing the stored carbon back into the system. As these threats have progressed, reclamation and erosion efforts have focused on restoring saltmarsh systems.

The overall goals of this study are to determine the contribution of allogenic and autogenic carbon to the restored wetland system. The specific objectives of this study include (1) determining the composition of the suspended sediment within the coastal watershed; (2) quantifying the organic carbon burial rate of belowground biomass and soils in the restored wetlands and a natural wetland; and (3) comparing soil organic carbon storage of the restored wetland to a natural wetland to determine the future carbon sequestration potential of a restoration project. We hypothesize that marsh thickness increases with restoration age, and carbon burial rates of restored wetlands are less than that in natural wetlands.

2. METHODS

2.1 Site Description

The North River Wetland Preserve (NRWP) is one of the largest wetland restoration projects of its kind in the United States. The construction of marshes typically involves mechanically grading the land such that the planting substrate creates a "carbon horizon" which contains essentially no soil organic matter, and serves as a marker of the time the wetland was constructed (Craft et al., 2003). The NRWP restoration stripped the original soils away to follow elevation designs, and while no soil was replaced, some higher elevation areas may have retained some of the original soils prior to construction. Carbon stocks from a natural wetland and the three restored wetland sites were examined. We used the chronosequence approach to assess ecosystem development of the constructed wetlands at NRWP. This approach relied on studying sites that have similar environmental conditions, but differ only with respect to their age. The obvious strength of this approach was that it "compresses" time, avoiding the need for long-term repeated measurements on a single site. A limitation of the approach was that, because of variable disturbance histories (drought, hurricane), differences among sites may be incorrectly attributed to ecosystem development rather than past disturbance events (Craft et al., 2003). Because the wetlands are relatively young (<15 yr) and in proximity, we are aware of construction practices and the disturbance history of the three sites. In previous research, inundation and vegetation have led to differences in the rate of carbon burial. To reduce this variability, the wetlands were paired with a nearby natural saltmarsh to serve as a comparison against which to measure carbon sequestration of individual restored wetlands. The carbon storage in natural wetlands provided a benchmark to estimate the carbon sequestration potential of restored wetlands.

2.2 Sample Collection and Analysis

Soil processing consisted of measuring sediment deposition and accumulation and storage of soil organic carbon. Sediment cores were collected in each marsh to measure sediment deposition. Suspended particles captured in sediment traps measured sediment flux in the channel that may be available for deposition. Two transects of four sediment cores (1-m soil auger) per marsh were collected for analysis (Fig. 1). Marsh thickness was measured from the surface to the identifiable basal contact of the marsh peat, identified as the top of a sand and/or clay substrate and indicated the initial marsh surface at the time of construction. Sediment cores from the natural wetland were collected and subsampled in 5-cm depth increments. Each core subsample was homogenized manually into a composite sample and the percent organic matter

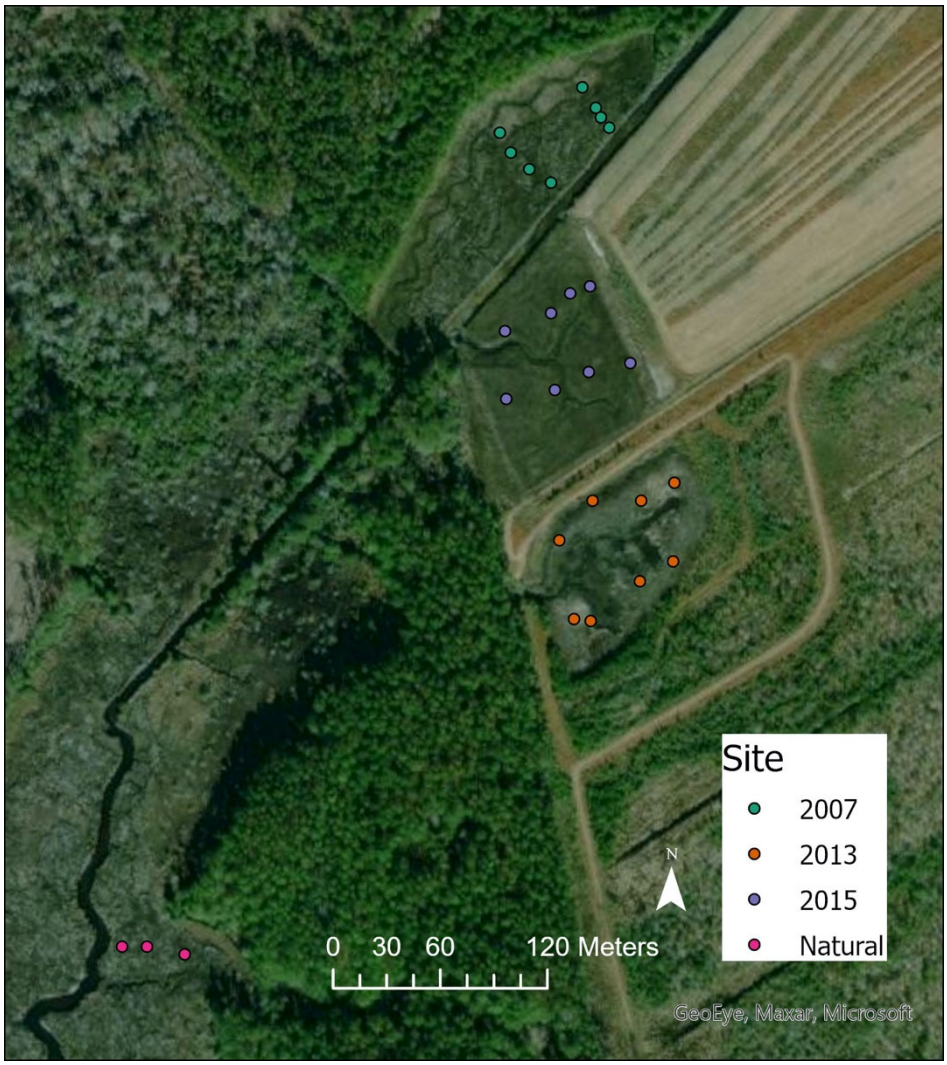

Figure 1. Core sample locations in four wetland sites.

was measured through loss on ignition. Percent organic matter was covered to percent carbon using the relationship published by Craft et al. (1991). Accumulation rates were based on the assumption that the base of the marsh represents the time that the restoration project was completed, and that organic carbon was uniformly distributed throughout the restored wetlands. Assuming the natural saltmarsh was accreting at the rate of sea-level rise (3mm per year), we analyzed the top 5 cm of the natural marsh for carbon and attributed 16.6 years as the age of that 5-cm interval (Kemp et al., 2009).

Sampling the total suspended sediment was critical to our understanding of carbon transport through the wetland system. We deployed sediment traps in the outflow channel from Open Grounds Farm to quantify the source and relative contribution of the suspended sediment within the coastal watershed. The time integrated mass sediment (TIMS) sampler was designed to trap sediment suspended in the water column. In this study, we utilized the modifications to the original Phillips et al. (2000) design, as described by Elliott et al. (2017). The modified design allows for the collection of suspended sediment in a bidirectional flow regime: tidal influences and the outflow from the upstream farmland. It utilized an 'L' shaped outflow tube to prevent backflow and was deployed in mirror pairs so that each sampler collected sediment in

one direction of tidal flow (Fig. 2). The body of the sampler was made of PVC pipe, 1-meter length, sealed using end caps, and positioned ~0.5m off the ground. The samplers were completely submerged at all times, and water level data (hydrology group) allowed us to account for differences in sediment flux according to tidal influences.

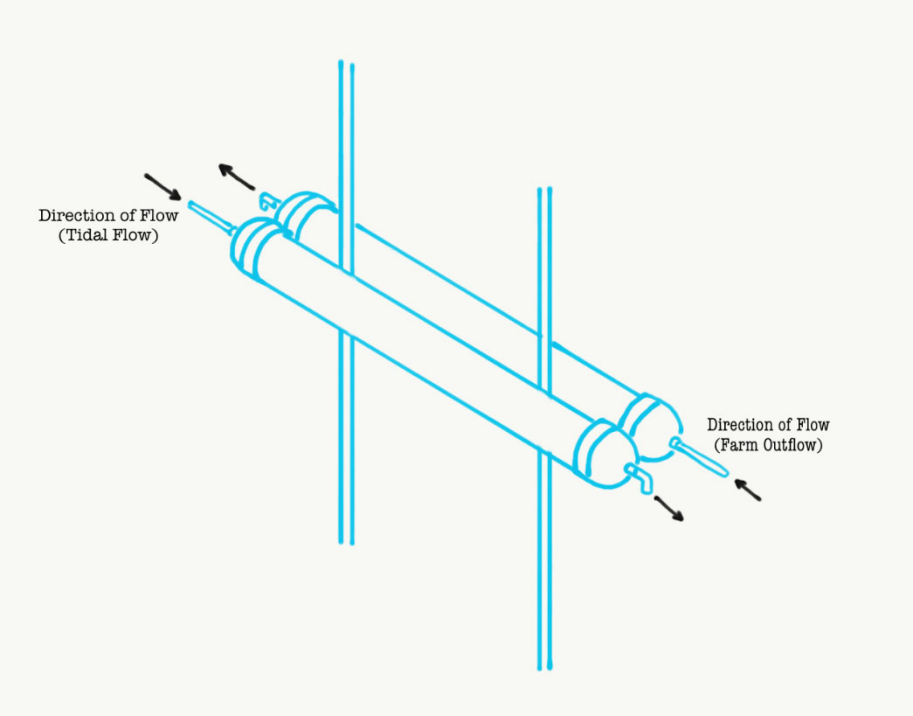

Figure 2. Three-dimensional view of bi-directional sediment trap, demonstrating SSL collection from upstream and downstream flow.

Sediment was collected at weekly intervals from two channel locations to calculate the total sediment loads. One location was near the farm outflow pipes, the other was farther down the same channel closer to the restored wetlands. Each TIMS sampler was deployed with one opening positioned toward the farm (ebb tide sampler) and the other with the opening positioned toward the estuary (flood tide sampler). Following each deployment, contents were collected for transport and the traps were redeployed. The suspended sediment was used to estimate the relative contribution of allogenic organic material supplied to the wetland by the channel. All analyses were expressed on a dry weight basis by drying subsamples for 24-36 hours at 70ºC then calculating loss on ignition after 4 hrs at 550ºC.

2.3 Calculations

To calculate the concentration of suspended carbon, we calculated the flow rate of the carbon and the flux of the carbon within the sediment trap. The flow rate was calculated at an upstream location using a flow-meter connected to an ISCO automated water sampler (nutrient group), and the flux of carbon was calculated from the weekly grams and the cross-sectional area of the sediment trap. These two values yielded a concentration in grams per cubic meters. We then integrated the water level above the average elevation of each marsh and multiplied by the marsh's respective area, to determine the total volume of water above each marsh over the sampling period. By assuming the sampling period was representative of a year, we converted to

volume of water above each marsh per year. The product of concentration and volume per year yields a value in grams per year for the entire marsh. Dividing by the marsh's respective area yields a flux in g/m^2/year of organic carbon suspended above each marsh per year.

3. RESULTS

3.1 Suspended Sediment Flux

Suspended sediment amounts varied by deployment location and flow direction. The amount of sediment decreased as the sediment moved further upstream or downstream, depending on the flow direction. The flood tide saw lower amounts of sediment overall compared to the sediment from the ebb tide (Fig 3). The percent organic matter demonstrated no significant difference between both site location and flow direction (Fig 4). The average percent organic matter across all samples was 23.7%.

Figure 3. TIMS sampler deployment location, the channel (red) and pipe outfall (blue) marked by the x. The bi-directional samples measured sediment amounts from the flood direction (flow from left to right) and the ebb direction (from right to left).

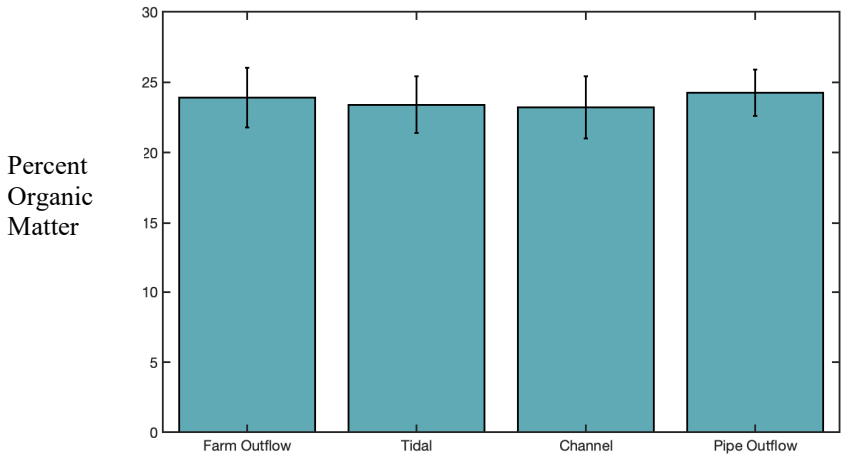

Figure 4. Percent organic matter by location and flow direction. From left to right, percentages are 23.2, 24.3. 23.9, and 23.2. Standard error is represented on all graphs. An ANOVA two factor test yielded a $P > 0.05$

To examine a possible connection between rainfall and sediment load, weekly sediment collection weights from the ebb tide were compared to total weekly rainfall. The data presented below is from September 9th 2020 to October 14th 2020 to include data from both TIMS sampler deployments (Fig. 4). Rainfall and sediment trends appear to follow a similar pattern. Sediment weights are averaged ebb tide flows for both TIMS samplers.

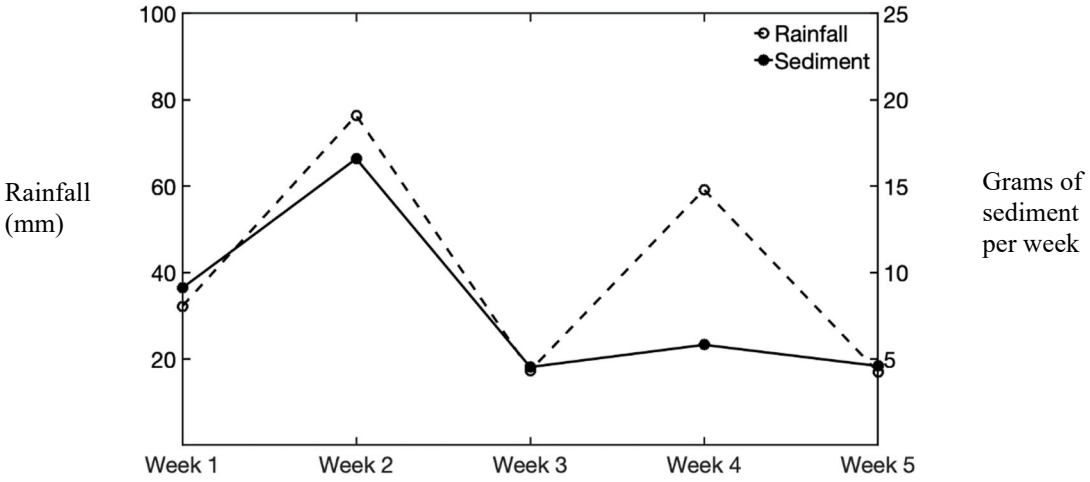

Figure 5. Weekly (9/9/20 – 10/14/20) amounts of precipitation from NOAA Station US1NCCR0020 (left axis) plotted with weekly amount of sediment from the farm outfall

3.2 Marsh Core Data

The percent organic matter (POM) of the natural marsh sampled (44.3%) was consistent with typical wetland percent carbon (Cahoon 2004, Wang 2019). The POM increased with marsh age, with no distinguishable difference between marshes restored in 2013 and 2015 (Fig. 5). Marsh thickness increased with marsh age, also demonstrating no difference between marshes restored in 2013 and 2015 (Fig. 5).

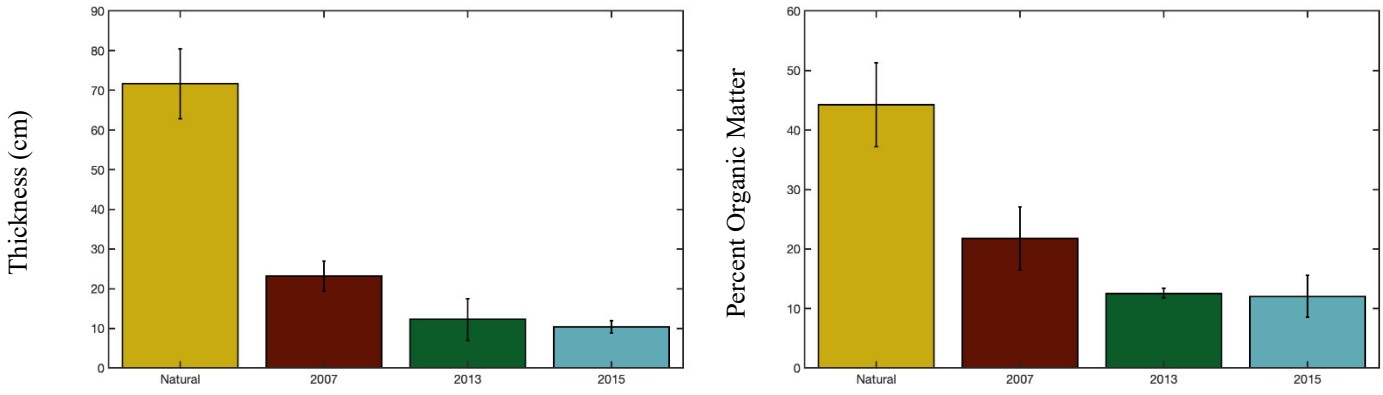

Figure 6. Average marsh thickness for each studied wetland (left). Average percent organic matter for each studied wetland (right). Standard error is represented for all data.

A significant positive correlation across all sites did exist between the thickness of the marsh and the POM (Fig. 6).

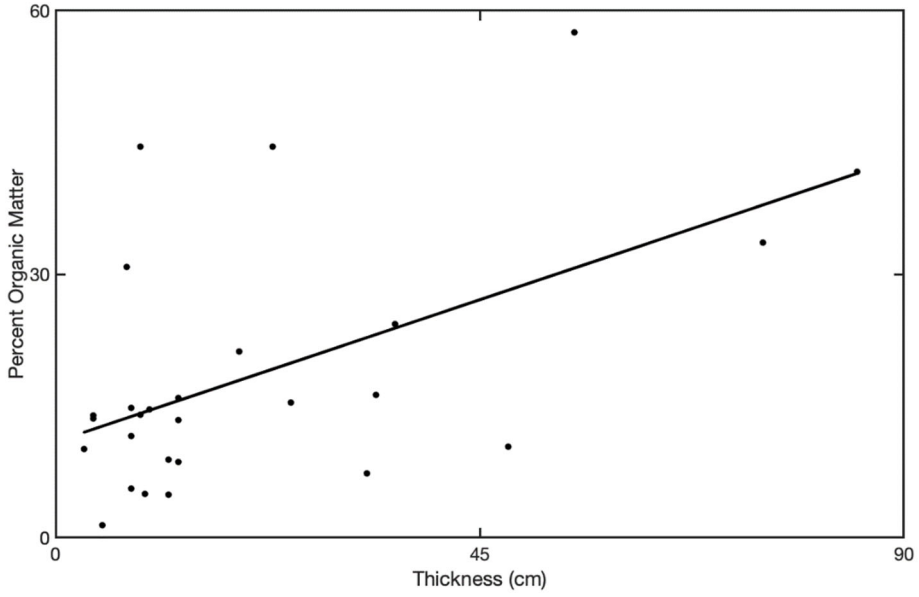

Figure 7. Thickness of the marsh compared to percent organic matter. Each point represents a core taken in any of the study sites. Linear regression resulted in a trend line with the equation: $y = 0.3593x + 10.926$, and $R^2 = 0.2971$, An ANOVA two factor test yielded a $P < 0.05$

3.3 Carbon Burial Rate

The carbon burial rate is measured as the flux of carbon into the marsh (g/m^2/year). The natural marsh's burial rate was 250 $g/m^{2/}$year. The restored marshes burial rates were an order of magnitude larger than the natural marsh, with values of 2559, 1276, and 1961 $g/m^{2/}$year for the marshes restored in 2007, 2013, and 2015, respectively (Fig 8).

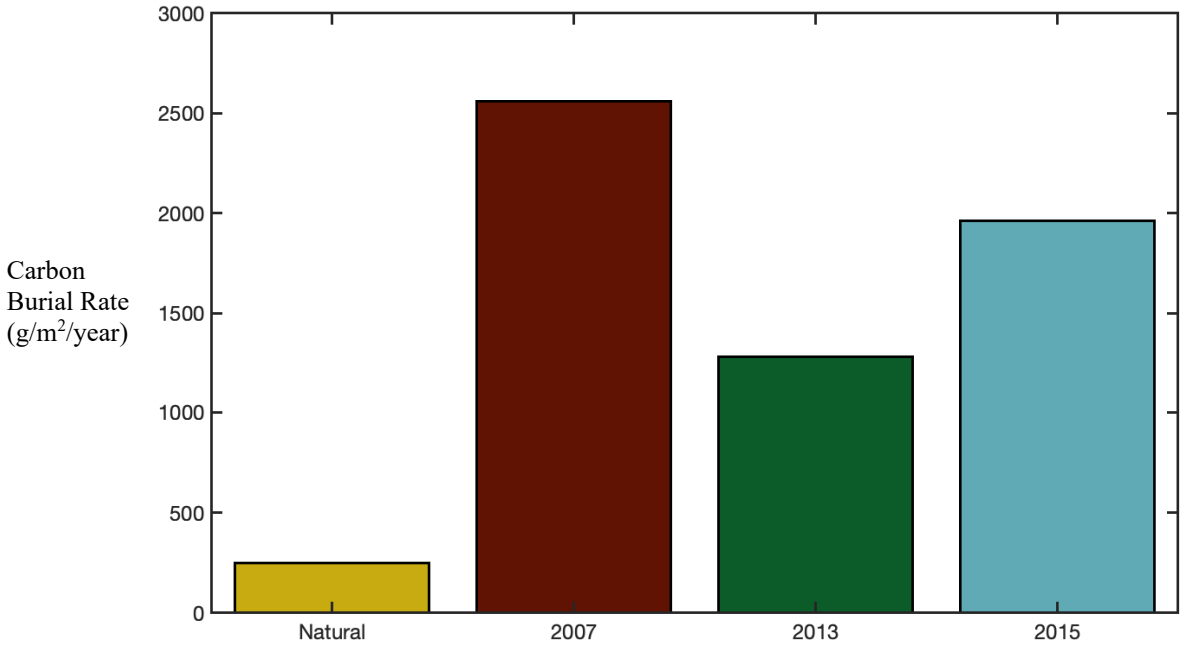

Figure 8. Carbon Burial Rate of each marsh measured in g/m^2/year.

3.4 Carbon Content Above Marsh

Based on the concentration of carbon in the water column and total volume of water above the wetland on a yearly basis, the suspended carbon above the marsh was determined for each of the restored sites. High values of carbon content above restored wetlands were found across sites (Fig. 9).

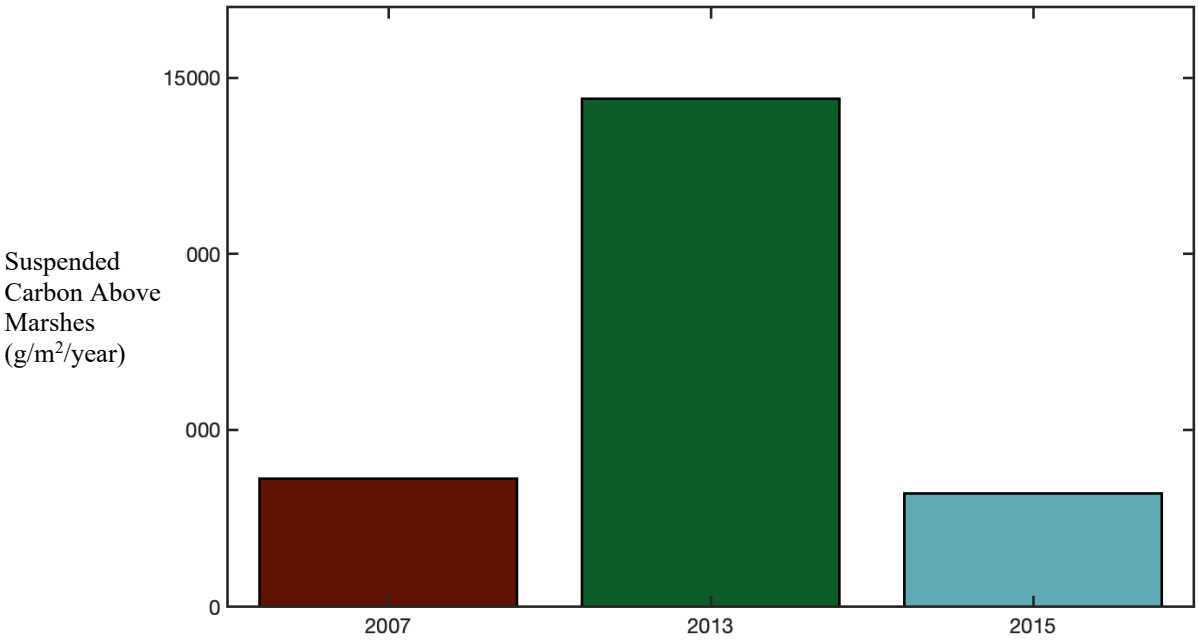

Figure 9. The annual suspended organic carbon for each restored wetland, measured in g/m^2/year. From left to right, values are 3625, 14410, and 3197 g/m^2/year

4. DISCUSSION

Our TIMS were effective at capturing large scale allogenic carbon and sediment dynamics. The flow dynamics of the channel are not great enough to overcome the settling rate of particles. When the flow was tidally dominated in the flood direction, sediment collection was greatest at the channel sampler and decreased upstream to the farm outflow sampler as particles settled out (6.5g/week for channel flow; 5.45g/week for farm outflow). In ebb flow, sediment collection was greatest at the farm outflow and decreased with distance down the channel as particles settled out (8.14 g/week at farm outflow; 7.68 g/week for channel). The amount of sediment collected is dependent on location and flow direction, with total amounts decreasing with distance from source of flow In general, sediment collection was greatest in the ebb direction, suggesting that the farm outflow adds a significant amount of sediment to the system. The TIMS was accurate in capturing larger particles within the water column but fine particles with a settling velocity greater than 50 m/day were unlikely to be captured. Fine particles are proportionately higher in organic carbon, suggesting that our sampling method underestimates carbon content in the system due to our inability to collect these particles. The TIMS were unable to show where deposition occurred, whether within the channel itself or within the wetland during inundation periods.

There was no statistical difference in the organic matter content across the flow direction and TIMS location, despite the range of sediment collection between the sites. The high carbon content in the collected sediment samples led us to hypothesize that suspended sediments originated from the farm. Comparison of the weekly rainfall values to the weekly ebb tide sediment amounts demonstrates a similar trend that increases of rainfall lead to increases in

suspended sediment. These findings support our post-sampling hypothesis that the source of suspended sediment in the water column is runoff from the adjacent farmland carrying high amounts of carbon. However, future research is necessary to determine the source of suspended sediments. Monitoring the carbon content and amount of suspended sediments closer to a farm outfall at shorter intervals, in addition to shorter interval precipitation data, could determine if runoff from the farm is the primary source of allogenic carbon. Researching deposition, specifically the rate and location of deposition, is important in determining the wetlands success in removing particulate matter from the water column (Venterink et al. 2006). Future work should gather suspended sediment data at shorter intervals within each marsh, especially during inundation.

The chronosequence approach had limited success in evaluating carbon sequestration. Marsh thickness correlated with the percent organic matter, and the natural marsh exhibited the highest POM based on marsh thickness. The 2007 marsh was the thickest among restored sites and contained the highest POM. The 2013 and 2015 wetlands demonstrated no statistical difference in POM, suggesting that the relatively small age difference between the 2013 and 2015 restoration sites may be the cause for the similarity between the two sites. The relatively recent restoration of the 2013 and 2015 sites may also explain the similarity between the two sites. There may be an initial lag time before the restored ecosystems begin to provide ecosystem services like sequestering organic matter.

The natural marsh's carbon burial rate was calculated by using the top 5 cm of the core. Based on marsh accretion due to sea level rise, the top 5 cm will represent 16.6 years of marsh accumulation (Kemp et al. 2009). We used this metric to date the natural marsh and compare the yearly carbon burial rate to that of the restored marshes. The natural marsh had a burial rate of 250 g/m^2/year, which is consistent with similar studies of coastal wetlands (Cunningham et al. 2016). We found the restored marshes carbon burial rates to be an order of magnitude higher than that of the natural marsh. This finding contradicts our hypothesis, and previous studies showing restored marshes do not sequester carbon at a significantly greater rate than a natural marsh (Drexler et al. 2019). Therefore, we hypothesized that the high carbon burial rates of the restored wetland are due to the high carbon content of suspended sediments in the channel. The tidal regime and sediment input from the channels allow the burial of carbon through both deposition and congregation of sediment on plant stems. The continual flooding of the restored marshes contributes to the overall allogenic carbon input to the system. Analysis found a high amount of carbon above the marsh, though the total contribution of suspended sediments to this unknown. The carbon contribution above the surveyed marshes is a similar or higher magnitude than the carbon burial rates observed in the same marshes, supporting our hypothesis that high carbon burial rates are a result of high carbon content in the suspended sediments.

When determining the contact point of the sediment cores, we assumed that there was no soil left before restoration. During construction, the farmland soils were stripped away to follow elevation designs for the restored wetlands, but areas of higher elevation may have retained some of the original soils prior to construction and restoration. Our assumption of a clean and defined carbon horizon may result in an overestimation of POM if the farmland sediments are encapsulated in our cores. Unfortunately, we have no way of determining what proportion of our core samples may retain this original farmland soil. The 2015 marsh had the highest elevation points and is most likely to have retained soils from the previous farmland state. Therefore, this marsh has the greatest possibility for overestimation of POM.

5. CONCLUSION

The restored wetlands show great potential for future carbon sequestration. Soil organic carbon is ideal for describing the development of salt marsh structure and function following marsh construction. Sedimentation from upstream and downstream sources were found to be primary sources of allogenic carbon; both the tidal component and farm outfall supply organic rich sediment. Carbon burial rates were extremely high in the restored wetlands when compared to the adjacent natural wetland. We suspect the suspended sediment contributes to these high carbon burial rates, though future monitoring of the site is needed to determine the carbon burial rate remains high as the marsh's age. Overall, we find that the restored wetlands are effective in carbon sequestration.

CHAPTER 3: Water Quality

1. INTRODUCTION

As the human population has increased with the development of advanced technology, environments have been degraded to support this ever-growing population–leading to a loss of natural resources and habitats. Restoration projects have been implemented to counteract this degradation of Earths' ecosystems because they help preserve biodiversity, maintain natural ecosystem services like carbon sequestration, and provide jobs to those in need (De Groot, 2013). In 1999, a restoration project was performed on the 6,000-acre North River row-crop agricultural farm to establish a restored wetland, the North Carolina Coastal Federation's North River Wetland Preserve (NRWP), that would help to provide the local, coastal environment of Carteret County, North Carolina with habitats for native species, protection from climatic events like hurricanes, and cleaner water (Woodward and Wui, 2001). The improvement of water quality in the North River due to the restoration project would allow more shellfish, a valuable water crop, to be harvested and reduce the risk of marine infections/diseases for recreational purposes. Thus, we conducted an observational study on the NRWP to evaluate the effectiveness of the restored wetland on water quality. Specifically, we examined the wetland's ability to improve water quality by quantifying concentrations and loads of fecal indicator bacteria (*E. coli* and *Enterococci*), *Vibrio* species (*V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus*), and Total Suspended Sediments (TSS) from its aquatic outfalls. Because the NRWP is adjacent to Open Grounds Farm, one of the largest farm operations East of the Mississippi, we were able to use it as a proxy to compare the bacterial loads at the restored wetland to the loads from the wetland's previous state (row crop agriculture). Additionally, the NRWP drains into both the North River and Ward Creek, estuarine bodies of water in Carteret County, North Carolina, which contains environmentally and commercially valuable shellfish. Through an analytical study, we also examined how the restoration project impacted fecal coliform concentrations within the North River and Ward Creek to gain a better understanding as to how effective the restoration has been on the water quality of the estuaries. In general, the objective of this study is to determine if the restoration of the NRWP reduced the concentrations and loads of fecal indicator bacteria, *Vibrio* species, and TSS in runoff from Open Grounds Farm and how these possible reductions may correlate to improved water quality in both Ward Creek and North River.

The quantification of concentrations and loads of pathogenic bacteria and TSS in the NRWP would help to increase the scientific community's knowledge as to how restored wetlands benefit their immediate aquatic environments. Currently, there has not been much research conducted on *Vibrio* removal in wetlands, but it has been found that constructed wetlands are able to reduce the concentration of *Vibrio* species when contaminated water flowed through a wetland treatment (Abdulla et al., 2007). Thus, we hypothesized that the NRWP would have lower loads of *Vibrio* species than the farmland. In terms of TSS, Kadlec (2003) determined that approximately 67% of TSS was removed by wetlands based on the median values of 21 wetland systems (Kadlec, 2003). Multiple studies have also found similar results with wetlands removing TSS at high rates. For instance, Knight and his associates found that wetlands can remove 53% of TSS concentrations–illustrating how effective wetlands are at removing TSS from aquatic systems (Knight et al., 2000). Hence, it can be hypothesized that the restored wetland outfalls would have lower loads of TSS than the farmland outfalls. However, it should

be noted that Jordan and his colleagues did not find a significant removal of TSS by a restored wetland in Maryland that had farmland runoff flowing through it (Jordan et al., 2003).

The examination of long-term trends in fecal coliform concentrations in both the North River and Ward Creek along with the quantification of fecal indicator bacteria loads in the NRWP can help us determine how effective the restoration project of North River farm has been on the water quality of Morehead City, North Carolina. Shellfish, vital marine organisms that help to purify water bodies, are found throughout the North River and Ward Creek in Carteret County, North Carolina where they can be harvested for human consumption. However, shellfish containing more than 1,000 *E. coli* per gram cannot be legally harvested (NSSP, 2017). The restoration of North River farm into a restored wetland, however, could possibly play an important role in reducing fecal coliform concentrations over a long temporal period–allowing more shellfish to be harvested. Previous research suggests that constructed wetlands are effective in decreasing fecal indicator bacterial loads by removing approximately 90 to 99% of total coliforms, *E. coli*, and *Enterococci* (Kaliakatsos et al., 2019; Kadlec, 2003). Furthermore, constructed wetlands with a variety of vegetation have been found to have a reduced amount of fecal indicator bacteria species as opposed to wetlands lacking vegetation (Wu et al., 2016). Therefore, we hypothesized that the restored wetland would have lower loads of fecal indicator bacteria than the farmland because wetlands are effective at removing fecal coliforms and the NRWP has a wide variety of vegetation as opposed to the farmland. Furthermore, we hypothesized that fecal coliform concentrations are decreasing in both the North River and Ward Creek due to the establishment of the NRWP because wetlands have been found to reduce concentrations of fecal indicator bacteria species as shown by the studies discussed previously. It should be noted that none of the studies previously mentioned discussed the effects of restored wetlands on the load of fecal indicator bacteria and *Vibrio* species, which means that the research outlined below is original work.

2. METHODS

2.1 Physical Conditions and Water Sample Collection Methods.

The North River Wetland Preserve has multiple outfalls that drain into the North River and Ward Creek. A few of these outfalls receive runoff from the adjacent row crop farm operation while others receive runoff from the restored wetland. In our study, we conducted spatially separated sampling at two of the farm outfalls (FR) and at two of the restored wetland outfalls (WRR). This was accomplished by collecting water samples at different points along the outfalls in one liter polyethylene bottles (Figures 1-5). The sampling sites needed to be spatially separated to qualitatively determine the distribution of the bacteria within the runoff. At first, we collected five water samples at each site, but we altered our methods due to time constraints by collecting three then two samples at each site that we labeled alphabetically (FR1A, FR1B, etc.). A field blank was also collected that was filled with deionized water. In terms of water velocity, we relied on ISCO samplers, automated water samplers, that were placed along the bank of the outfalls. A water-monitoring program was input into the samplers, allowing us to quantify the water velocity and height in the outfalls. Additionally, we determined the width of each outfall by using a measuring tape. Temperature and salinity were additionally collected by using an electronic thermometer at time of collection and an electronic refractometer once we returned to the University of North Carolina at Chapel Hill's Institute of Marine Sciences (IMS) laboratory, respectively.

2.2 Vibrio Methods.

The assessment of *Vibrio* accumulation in the reserve was conducted by using CHROMagar plates and vacuum filters to grow *Vibrio* colonies. Before the water samples were collected, we prepared at least 48 CHROMagar Vibrio (CAV) plates that contained around 5 to 7 milliliters per plate of the CAV media. The media was produced by dissolving 26.2 grams of CAV powder into 350 milliliters of distilled water, boiling and swirling the mixture to dissolve the formed crystals, and keeping the mixture warm in a 50°C water bath until the solution was ready to be poured into 50 by 9-millimeter sized Pall petri dishes. Once the water samples were collected, the samples from both the restored wetland and farmland outfalls were diluted based upon their salinity by pouring the raw water into 50 milliliter tubes containing 10x concentrations of Phosphate Buffered Saline (PBS). The amount of PBS solution mixed with the raw water from the restored and farmland outfalls depended upon the salinity values of the water samples, which is based on the research conducted by Brett Froelich and his colleagues on the Neuse River Estuary (Froelich et al., 2013). This dilution allows our *Vibrio* cultures to be comparable by creating an optimal number of colonies to be counted (30-80 colonies). These dilutions were then filtered in a vacuum manifold through 47-millimeter MCE Millipore filters and then were placed on the CHROMagar plates with no air bubbles to allow optimal bacterial growth. In total, there are six CHROMagar plates per water sample because we used three dilutions and duplicates to minimize analytical errors. After a 24-hour incubation period in a 37°C heated incubator, the bacterial colonies were counted. A colony is identified by whether or not it has a clearly defined center. These colonies are small enough that a magnifying glass is frequently necessary to count the plates. We counted three different species of *Vibrio* that are known to contaminate oysters and cause adverse symptoms in humans (*V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus*). The colonies are identified by their color with *V. alginolyticus* adopting a creamy yellow color, *V. vulnificus* becoming turquoise and *V. parahaemolyticus* becoming purple (Kaysner & DePaolo, 2004).

2.3 Fecal Indicator Bacteria Methods.

We used the IDEXX Quanti® tray method for determining *E. coli* and *Enterococci* concentrations. First, we injected ten milliliters of each water sample into ninety milliliters of deionized water that had been invertedly mixed with Colilert-18® and Enterolert™ media packets ("*Quanti-Tray System,*" 2020). After mixing the media and deionized water with our field samples, the media mixtures were placed and sealed into 49/48 IDEXX Quanti® trays by an IDEXX Quanti® sealer ("*Quanti-Tray System,*" 2020). Initially, there were between 40 to 48 trays of media mixtures for each date of sampling. This tray count included duplicates of our 8 samples and field blank from sites FR1, FR2, WRR1, and WRR2 to assess analytical error. However, we decreased our tray count to 34 trays due to time constraints previously mentioned (17 Enterolert™ and 17 Colilert-18®). The Colilert-18® samples were incubated for at least 18 hours (no more than 22 hours) at 35°C while the Enterolert™ samples were incubated for a minimum of twenty two hours (no more than 26 hours) at 41°C ("*Quanti-Tray System,*" 2020). If the capsules on the Colilert-18® tray were a cloudy yellow, then they were positive for total coliforms ("*Quanti-Tray System,*" 2020). In addition, the fluorescence of these capsules indicated the presence of *E. coli* when placed under a black light. In terms of *Enterococci* presence, if a capsule was fluorescent underneath the black light, then it was positive for the bacteria ("*Quanti-Tray System,*" 2020). After collecting data on fecal indicator bacteria species, we determined the

Most Probable Number (MPN) of bacteria through the use of an MPN calculator and multiplying the MPN values by a value of ten to account for the dilution of our water samples ("*Quanti-Tray System,*" 2020).

2.4 Total Suspended Sediments (TSS) Methods.

Total Suspended Sediment (TSS) measurements were accomplished by initially preparing and weighing glass fiber filters wrapped in aluminum foil squares on an analytical balance. Next, we filtered one-hundred milliliters of sample water through the previously weighted glass fiber filters in a vacuum manifold instrument. After our water samples had been filtered, we wrapped the filters back inside their designated piece of aluminum and placed them inside an oven to dry at 55 °C for a minimum of two days. When they finished drying, we reweighed the filters to determine the TSS of our water samples in grams by calculating the difference between the glass fiber filter's initial and final weight. We conducted duplicates of each water sample to characterize the analytical errors and minimize errors when reporting sample means.

2.5 Long Term Trends of Fecal Coliform Data Collection.

Through spatial computer analysis, we determined a temporal relationship between the establishment of the NRWP and fecal coliform concentrations (colonies/100mL) in both the North River and Ward Creek from data collected by the Division of Marine Fisheries (DMF). At first, we divided the North River into three different sections to represent the upper, middle, and lower portion of the river (Figure 6 and Table 1). This process was repeated on Ward Creek, but only two sections (upper and lower) were created (Figure 6 and Table 2). Next, we located the water sampling sites conducted by the DMF that were within our desired sections based on their latitude and longitudes, and we added the fecal coliform concentration data from these sampling sites to an Excel spreadsheet. If the NRWP had an effect on fecal coliform concentrations, then we would observe that the upper portions of the rivers would experience the greatest decrease in fecal coliform concentrations.

Table 1. Latitude and Longitude Ranges of Sample Sites in North River Sections

North River	Latitude-Longitude Ranges
Upper Section	34.79195742, -76.61926403 34.80613849, -76.61043845
Middle Section	34.77569212, -76.6178632 34.79195742, -76.6178632
Lower Section	34.75856989, -76.61891379 34.77569212, -76.61891379

Table 2. Latitude and Longitude Ranges of Sample Sites in Ward Creek Sections

Ward Creek	Latitude-Longitude Ranges
Upper Section	34.780578, -76.573922 34.786080, -76.569915
Lower Section	34.76495407, -76.58580358 34.77692551, -76.56747026

2.6 Analysis of Load Data.

The Microsoft program Excel helped us efficiently calculate both of our flow and load datasets. Initially, we input all of our collected data (MPN calculations of fecal indicator bacteria, *Vibrio* concentrations, cross-sectional area measurements, and TSS data) into Excel spreadsheets (one for each dataset). Next, we calculated the average flow over the whole study period by multiplying the collected water velocity data with the measured cross-sectional area of the outfall. The cross-sectional area was produced by multiplying the water height with the width of the outfall. After the flow data was calculated, we separately multiplied average concentrations of fecal indicator bacteria, TSS, and *Vibrio* species with the average flow data to produce our load datasets. Additionally, we divided the load data by the differing watershed areas so that the data could be comparable. Through Excel, we were able to produce the figures shown in the results section below (Figures 13-15; 22-24; 27).

2.7 Long Term Trend Analysis of North River and Ward Creek Fecal Coliform Data.

After dividing the North River and Ward Creek estuaries into segments, we analyzed the fecal coliform concentrations data at the stations in each estuarine segment by calculating averages of all the bacteria data collected within each segment every five years (Tables 1-2). This, in turn, produced two Excel scatter plots that demonstrate the relationship between time and average fecal coliform concentrations every five years in both Ward Creek and North River among the different sections we established (Figures 28 and 29).

Figure 1. Image depicting our four sampling sites for restored wetland (WRR) and farmland (FR) outfalls.

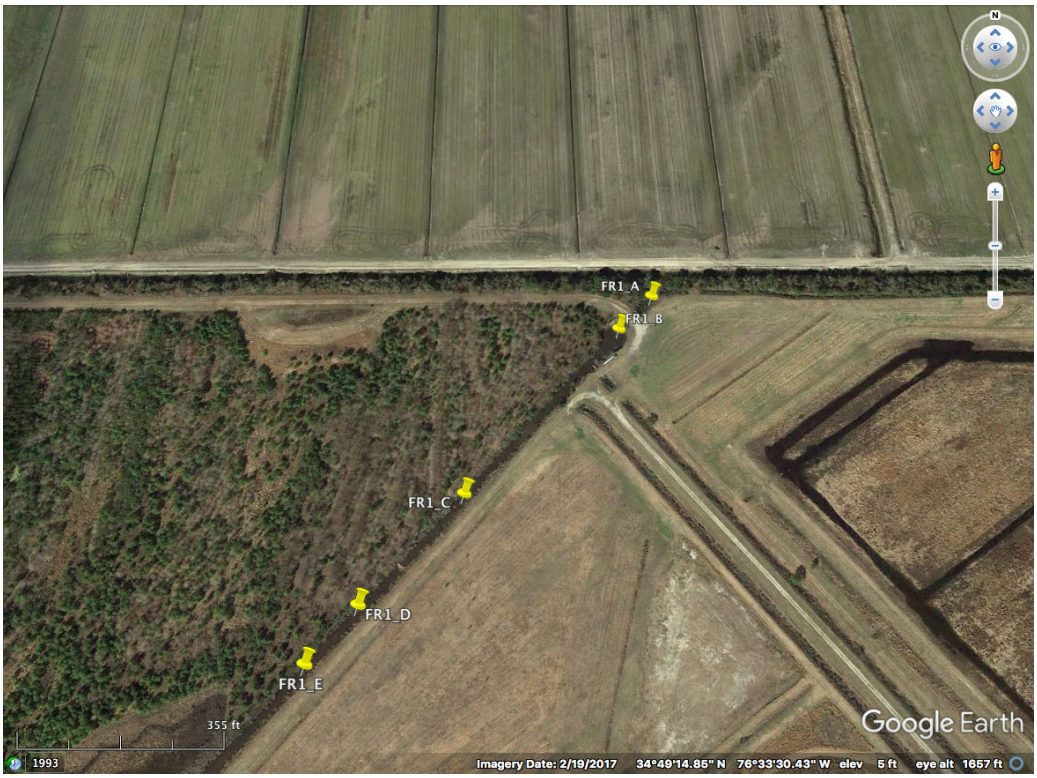

Figure 2. Image depicting the five sample sites from the first farmland outfall.

*Figure 3***.** Image depicting the five sample sites from the first restored wetland outfall.

Figure 4. Image depicting the three sampling sites from the second restored wetland outfall.

Figure 5. Image depicting the three sampling sites from the second restored wetland outfall.

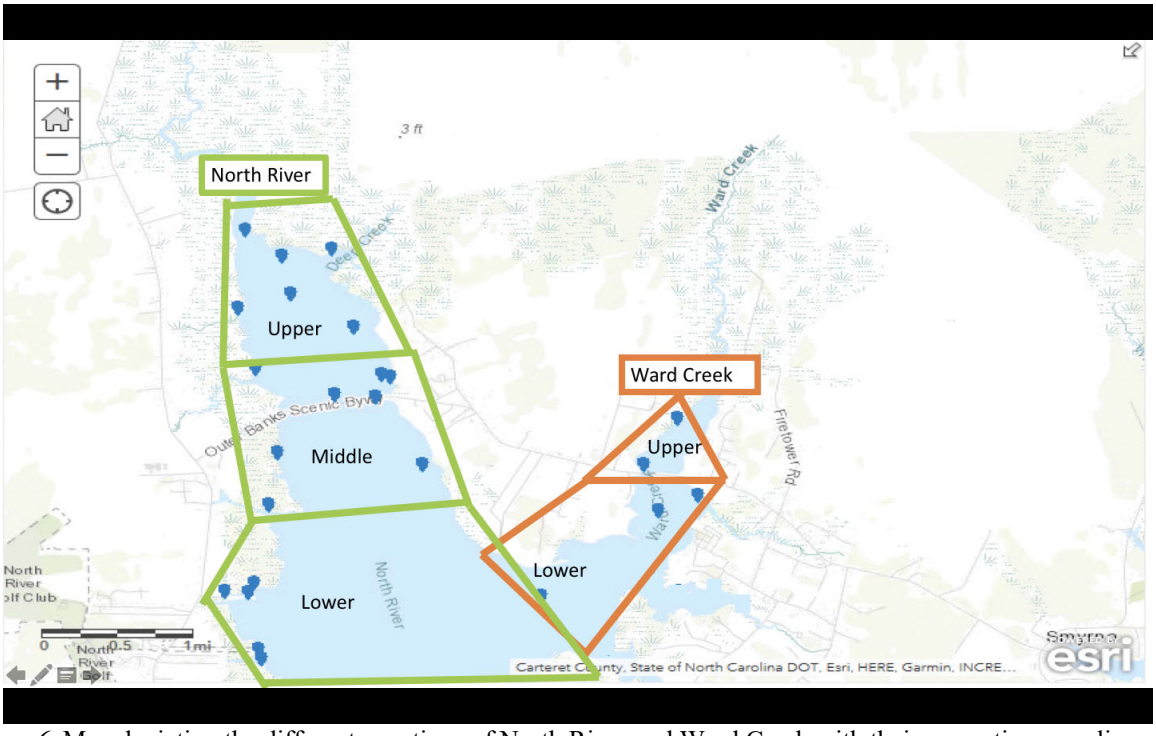

Figure 6. Map depicting the different zonations of North River and Ward Creek with their respective sampling sites.

3. RESULTS

3.1 Vibrio spp. Concentrations and Loads.

On average, Wetland 2 had the lowest average *V. parahaemolyticus* concentration while Farmland 1, the site with the highest average concentration, was about four times higher (Figure 7). The average *V. vulnificus* concentration of Farmland 2 was three times higher than that of Wetland 1; these sites possessed the maximum and minimum *V. vulnificus* concentrations, respectively (Figure 8). The average *V. alginolyticus* concentration at Farmland 1, the highest average concentration site, was about four times higher than that of Wetland 1, the lowest average concentration site (Figure 9). The daily concentrations of each *Vibrio spp.* showed interesting patterns. For example, the two Farmland sites expressed high levels of *Vibrio spp.* on September 9 (Figures 10-12). *V. vulnificus* concentrations in particular appeared to be low on most individual days, with one day at each site pulling up the average concentrations (Figure 11).

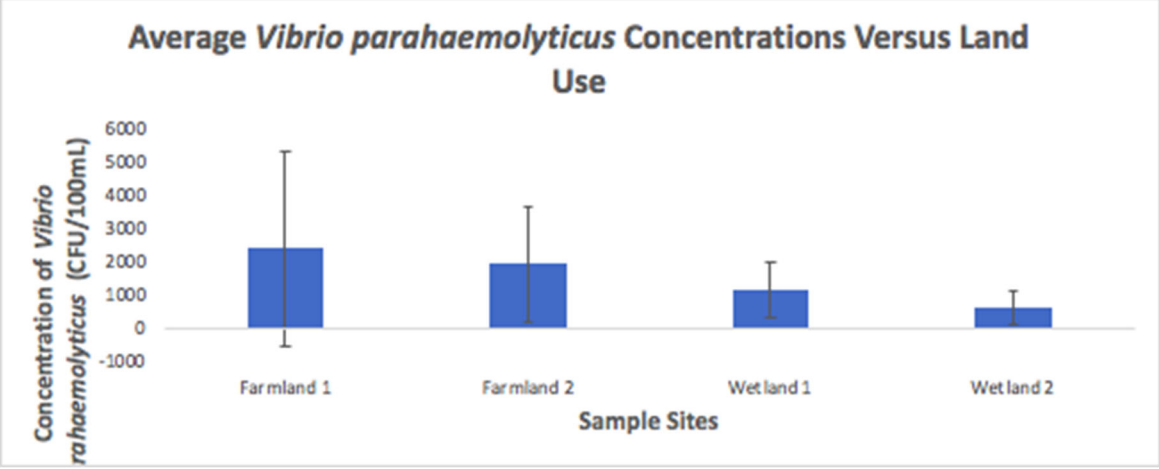

Figure 7. Concentrations of *V. parahaemolyticus* colonies averaged over the course of data collection

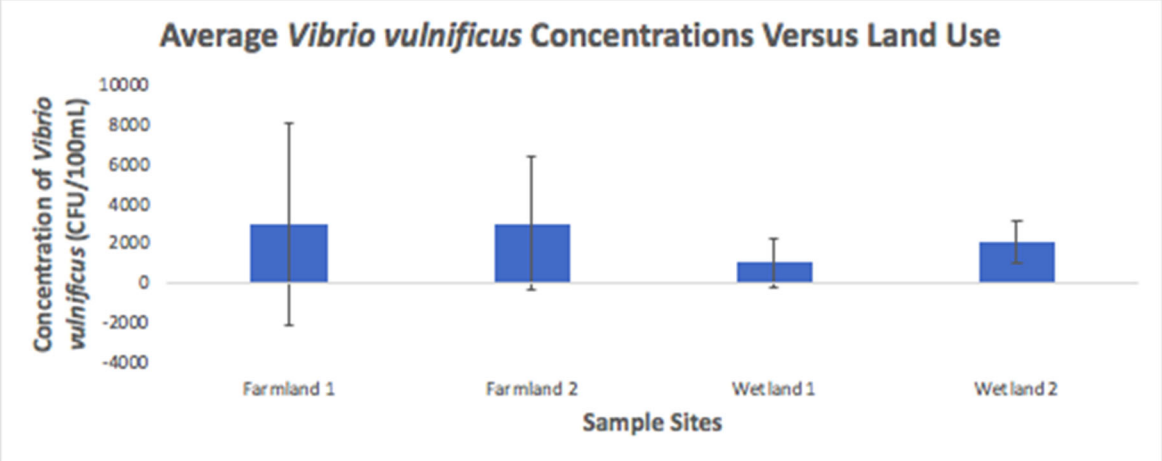

Figure 8. Concentrations of *V. vulnificus* colonies averaged over the course of data collection

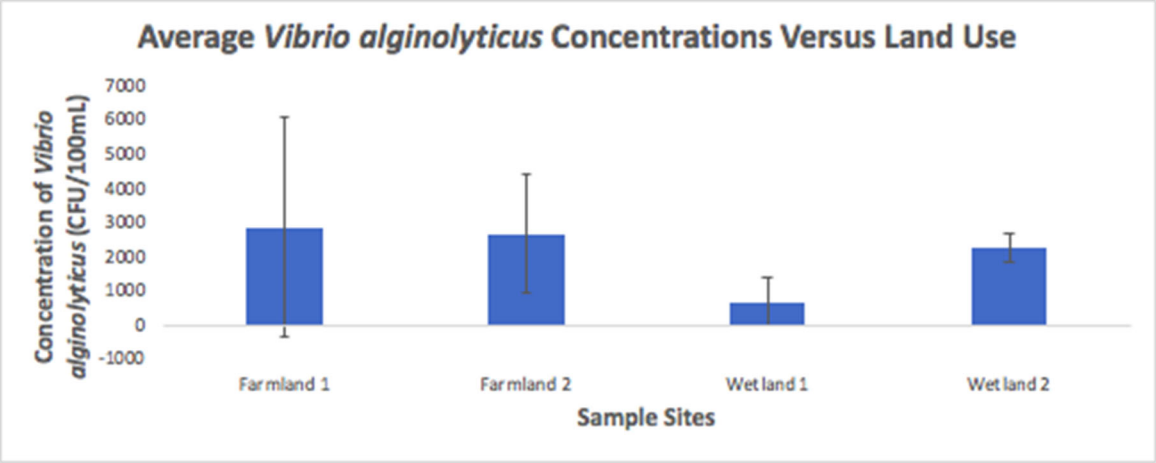

Figure 9. Concentrations of *V. alginolyticus* colonies averaged over the course of data collection

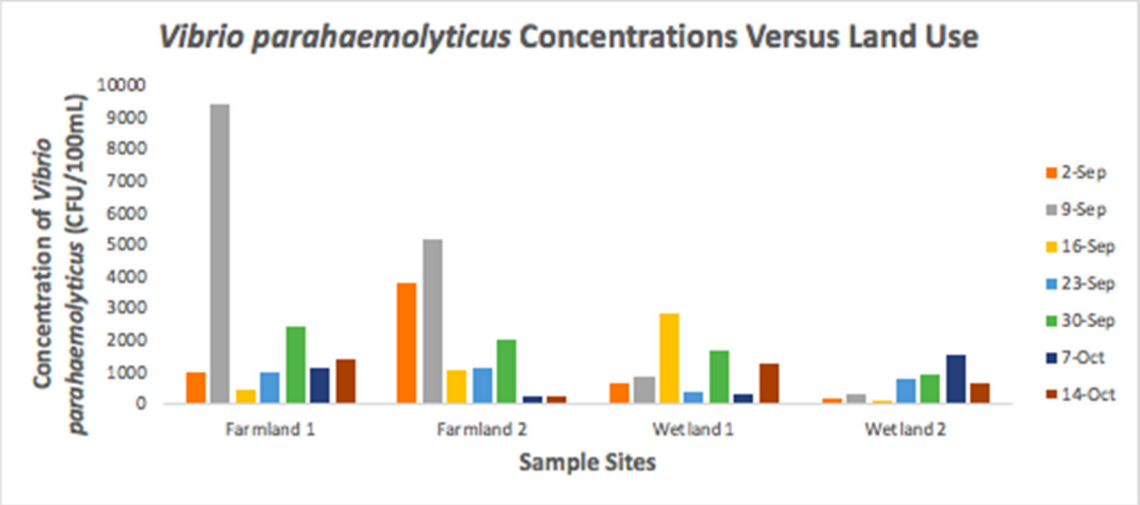

Figure 10. Concentrations of *Vibrio p.* colonies each day of sample collection at each of the four sample sites

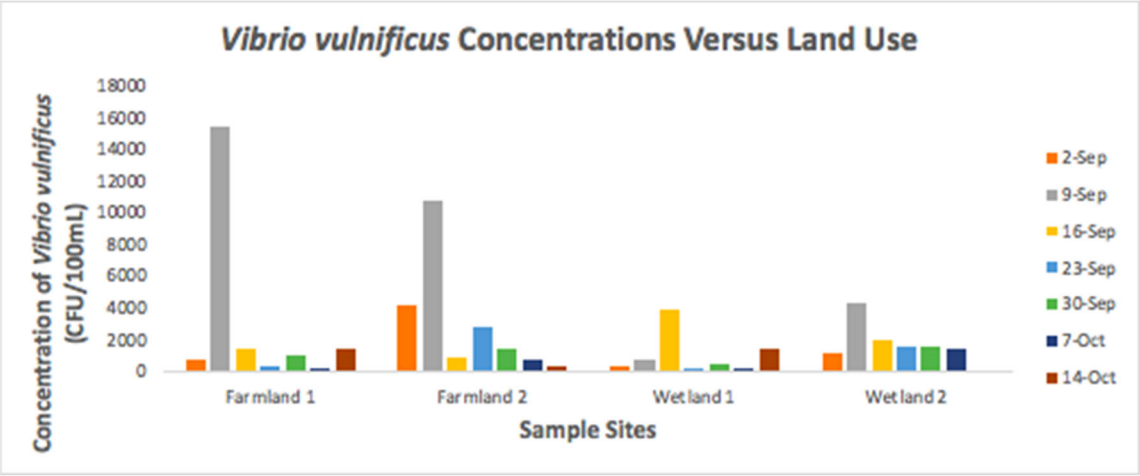

Figure 11. Concentrations of *Vibrio v.* colonies each day of sample collection at each of the four sample sites

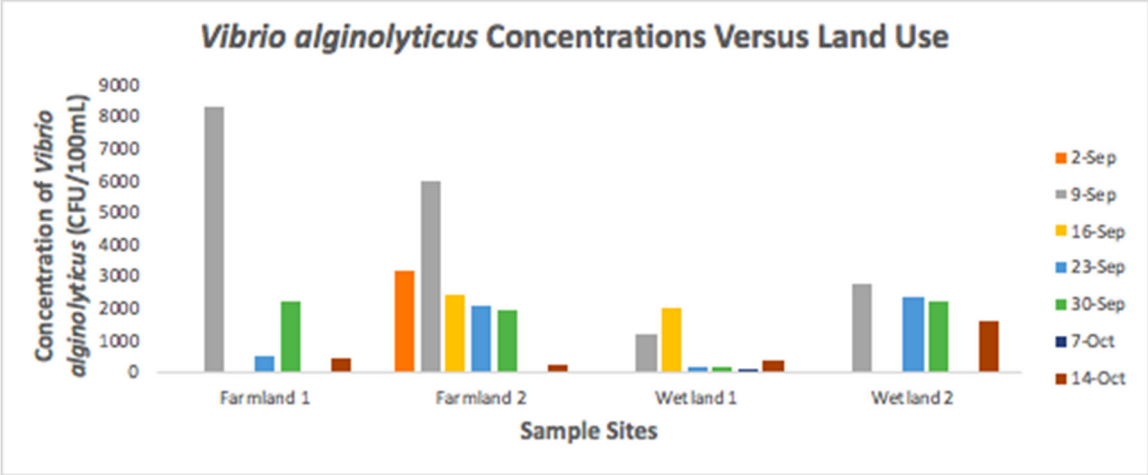

Figure 12. Concentrations of *Vibrio a.* colonies each day of sample collection at each of the four sample sites

V. parahaemolyticus load values were highest at Farmland 2, 15 times higher than the lowest at Wetland 1 (Figure 9). Wetland 2 did not experience water flow and consequently did not express bacterial loading, a pattern that follows in each bacterial category observed. The highest *V. vulnificus* load, Farmland 2, was about six times higher than the lowest at Wetland 1 (Figure 12). As for *V. alginolyticus*, the highest load came from Farmland 2 and the lowest came from Farmland 1, which was about nine times lower than Farmland 2 (Figure 15).

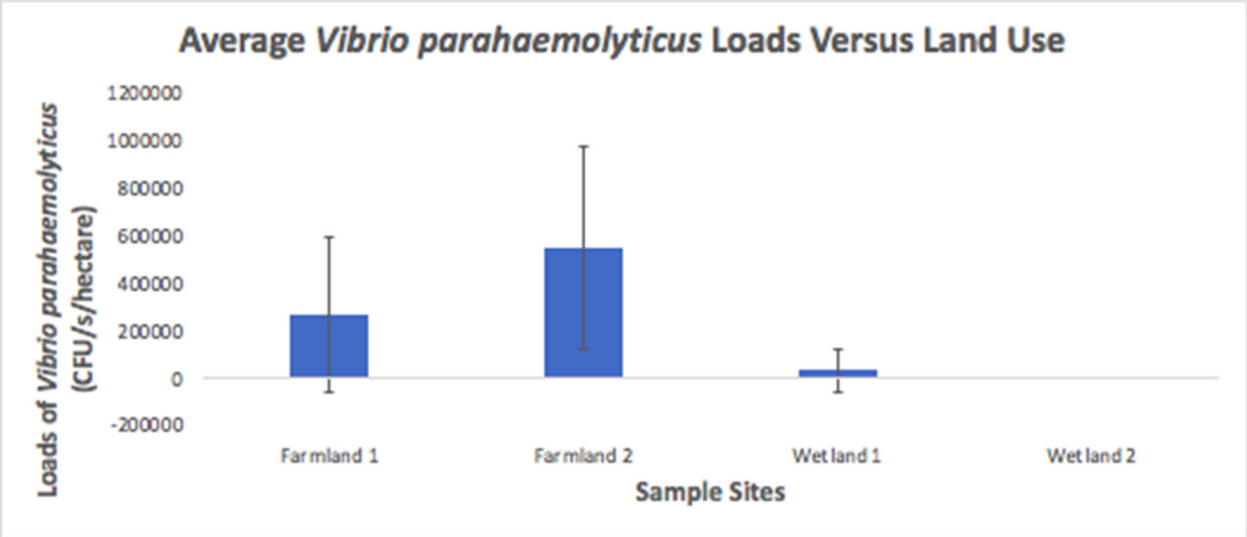

Figure 13. This chart depicts the average load of *V. parahaemolyticus* at each of the three sites that experienced water flow over the course of data collection and bars corresponding to each site's standard deviation.

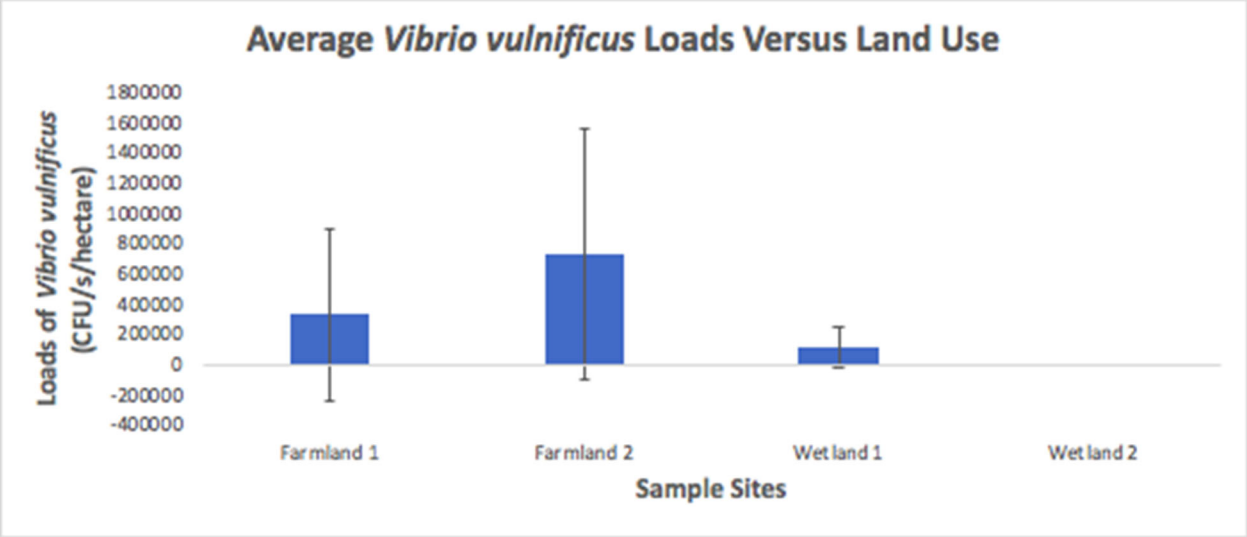

Figure 14. This chart depicts the average load of *V. vulnificus* at each of the three sites that experienced water flow over the course of data collection and bars corresponding to each site's standard deviation.

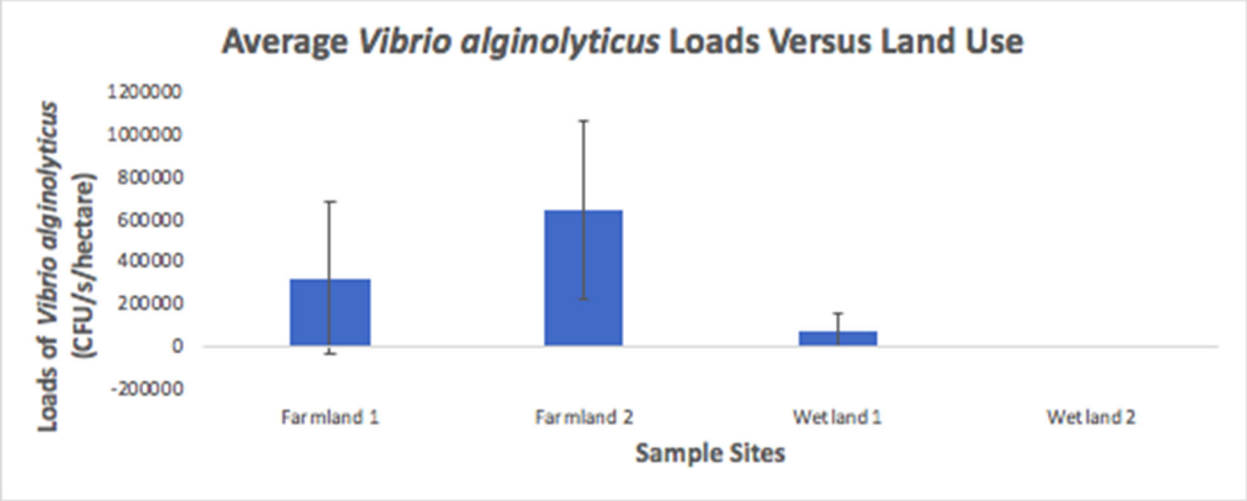

Figure 15. This chart depicts the average load of *V. alginolyticus* at each of the three sites that experienced water flow over the course of data collection and bars corresponding to each site's standard deviation.

3.2 Fecal Indicator Bacteria Concentrations and Loads.

The two farmland sites each displayed higher concentrations of *E. coli* than the two wetland sites (Figure 16). Furthermore, we found that the farmland outfalls had higher variance than the wetland sites. Wetland site 1 had a concentration of *E. coli* that was about four times higher than the other Wetland site. Farmland 2 held the highest average concentration of *E. coli*, about seven times higher than the lowest concentration at Wetland 2 (Figure 16). While the variation in the two Farmland sites and Wetland 1 were visibly high, Wetland 2 had low enough deviation that we can say with confidence that Wetland 2's *E. coli* concentrations were lower than Farmland 2 (Figure 16). As for *Enterococci*, we found that Farmland 1 had the highest average concentration value, followed by Farmland 2, then by Wetlands 1 and 2, respectively. Again, Wetland 2 maintained its low concentration (Figure 17). The average *Enterococci* concentration of Farmland 1 was 38 times higher than Wetland 2 (Figure 17). When we analyzed the concentration of total coliforms at each sample site, we found that Farmland 1 showed the highest average concentration, followed by

Farmland 2, Wetland 2, and Wetland 1 (Figure 18). Farmland 1's average concentration of total coliforms was about four times greater than that of Wetland 1 (Figure 18).

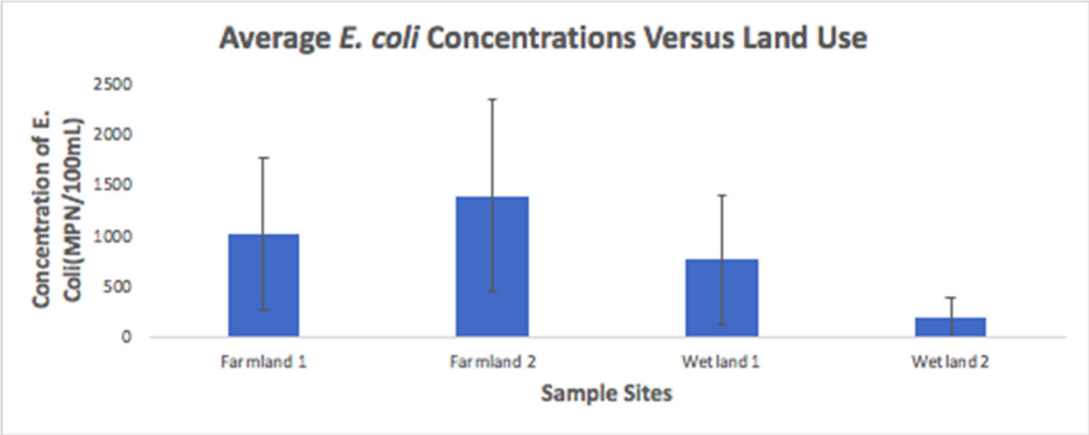

Figure 16. This chart displays the average concentration of *E. coli* found on our four sample sites and their associated standard deviations as error bars.

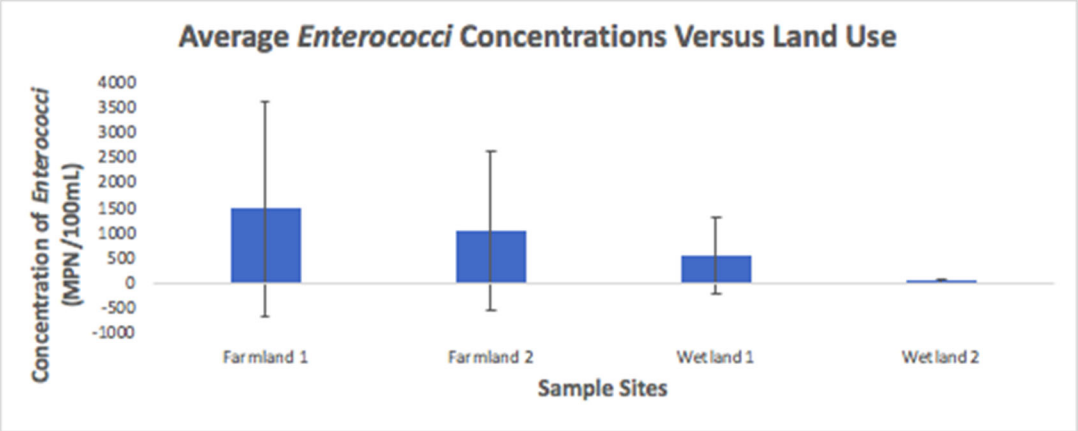

Figure 17. This chart displays the average concentrations of *Enterococci* found on our four sample sites and their associated standard deviations as error bars.

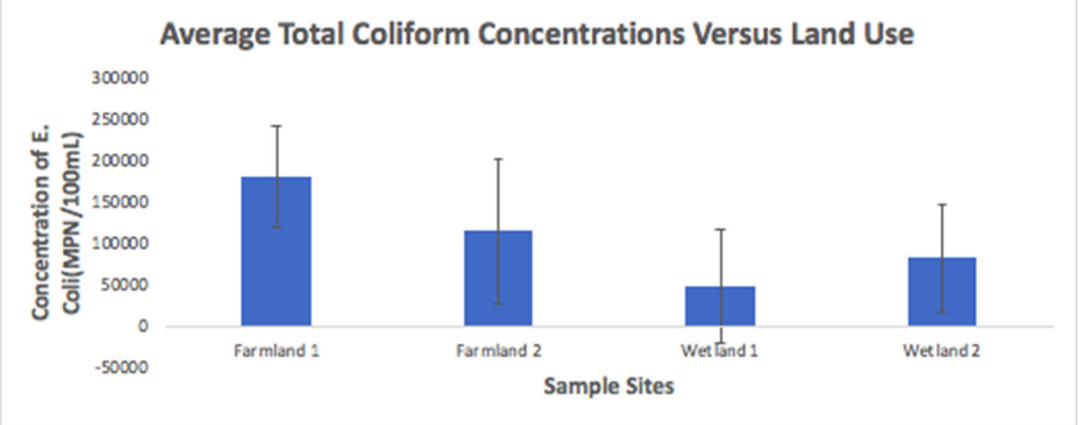

Figure 18. This chart shows the average concentrations of total coliform bacteria over the course of data collection at each site.

We also took the *E. coli* concentrations of each of the four sample sites on each day that sampling took place (Figure 19). We can see that the concentrations at the two Farmland sites were highly variable and that the concentrations of the two Wetland sites were fairly consistent (Figure 19). It is noteworthy that most days showed fairly low concentrations of *E. coli* with several days with abnormally high concentrations driving up the average values at the first three sample sites. When we observed the daily *Enterococci* concentrations at each sample site over the course of data collection, we found that the concentrations were low on most days with several days, such as October 14th and September 30th, showing massive *Enterococci* concentrations that pull the average values up at both Farmland sites and the first Wetland site (Figure 20). The Farmland sites experienced spikes in *Enterococci* concentrations on both of the mentioned dates while the first wetland site only saw abnormal values on October 14th (Figure 20). Finally, we found that the Farmland outfalls had consistently higher concentrations of total coliforms than the Wetland outfalls (Figure 21). October 7th, however, had low concentration values of total coliforms at all sites. Farmland 1 showed consistently high levels of total coliforms, while Wetland 1 showed only two days with high coliform levels, September 9th and 16th (Figure 20).

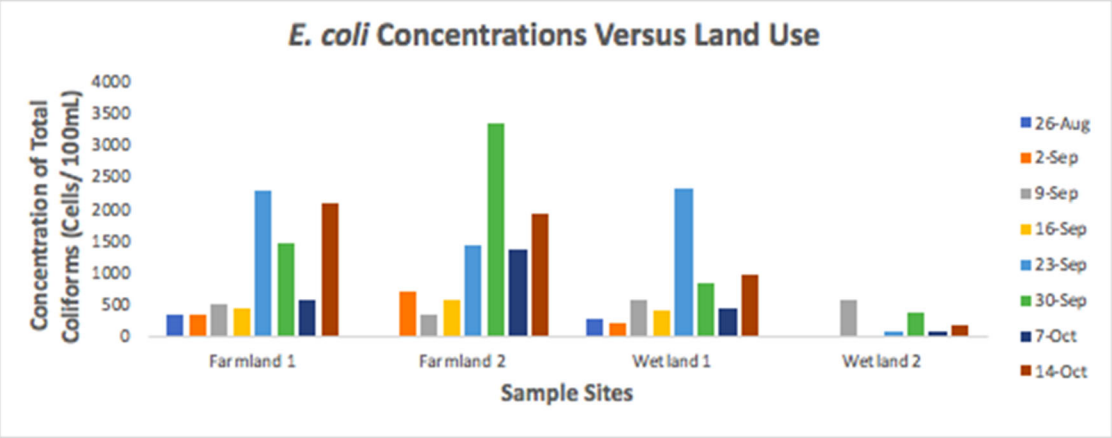

Figure 19. This chart displays the *E. coli* concentrations at each site on each day of sampling.

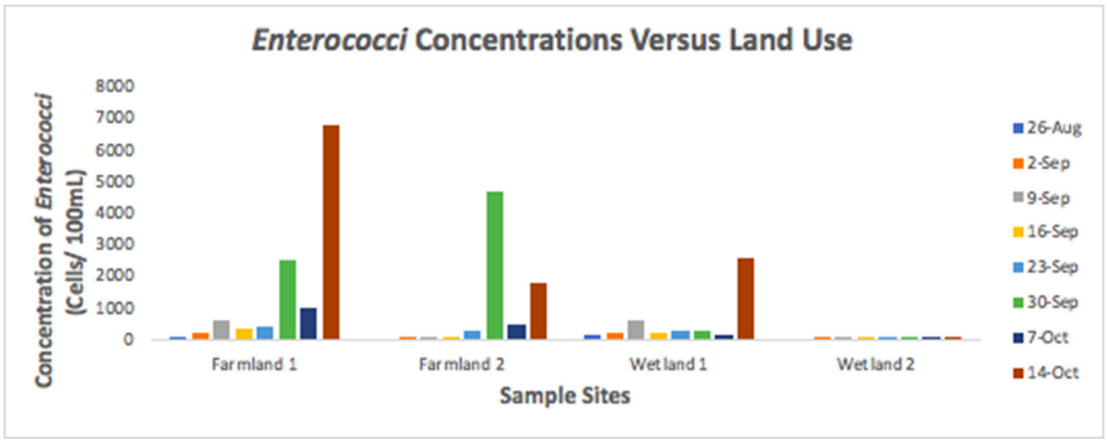

Figure 20. The *Enterococci* concentrations observed on each sampling date at each sampling site.

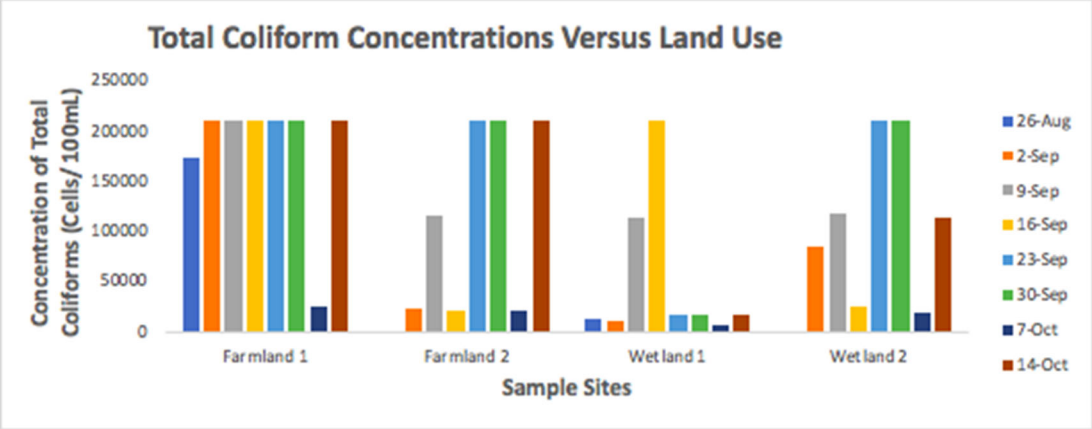

Figure 21. This chart shows the total coliform concentrations at each site on each date of sample collection; 200,000 cells/100mL represented the upper detection limit.

In terms of *E. coli* load values, Farmland 2 maintained the highest average load value, about four times higher than Wetland 1 (Figure 22). We also found that Farmland 2 possessed the highest average load value of *Enterococci*, about seven times higher than Wetland 1(Figure 23). According to the standard deviation bars, Farmland 2's lead in *Enterococci* load is statistically significant (Figure 23). Additionally, we found that the second Farmland site had the highest average load value of total coliforms, about four times greater than Wetland 1 (Figure 24).

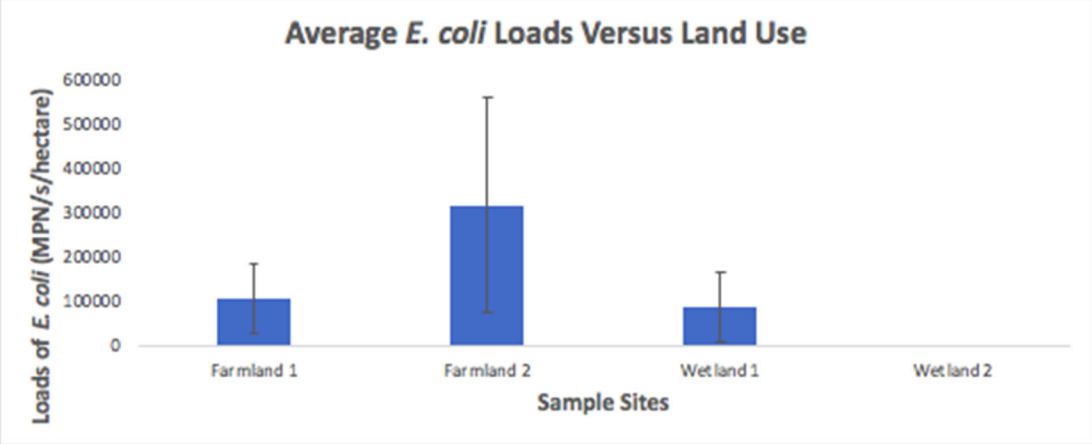

Figure 22. This figure demonstrates the average *E. coli* loads of the three sites that demonstrated any degree of water flow.

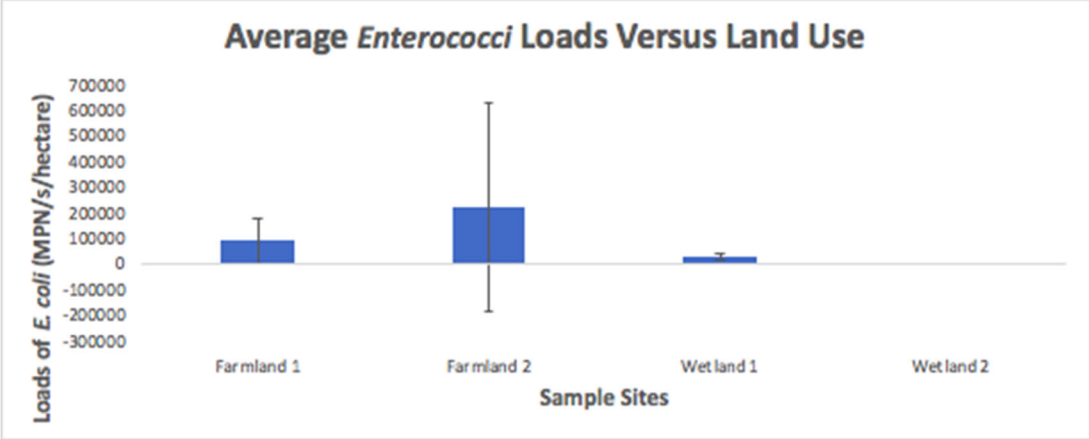

Figure 23. This figure demonstrates the average *Enterococci* loads of the three sites that demonstrated any degree of water flow.

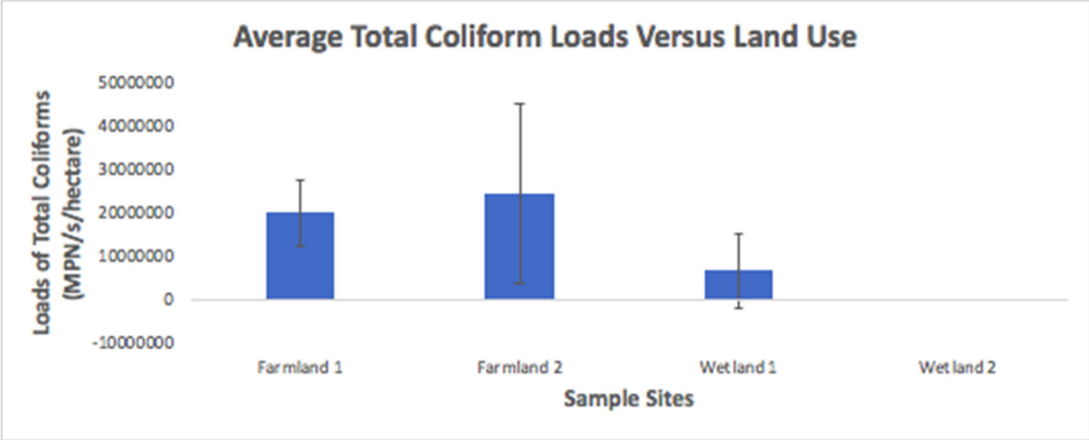

Figure 24. This chart shows the average total coliform loads of each relevant site along with error bars that account for standard deviation.

3.3 Total Suspended Sediments.

We found that Farmland 2 had the highest concentrations of Total Suspended Sediments while Wetland 2 had the lowest, with a threefold difference between them (Figure 25). The total suspended sediment concentrations on each day of data collection show that many of the individual days at each site had fairly low concentrations (Figure 26). Several days of extremely high sediment concentrations drove the average values up at the Farmland sites. The Wetland sites were consistently lower in concentration than the Farmland sites. The Farmland 2 site showed the highest sediment load of TSS while Wetland 1 showed the lowest load, with a roughly five-fold disparity between them. However, the average sediment load values were very similar, differing by mere milligrams per second per hectare.

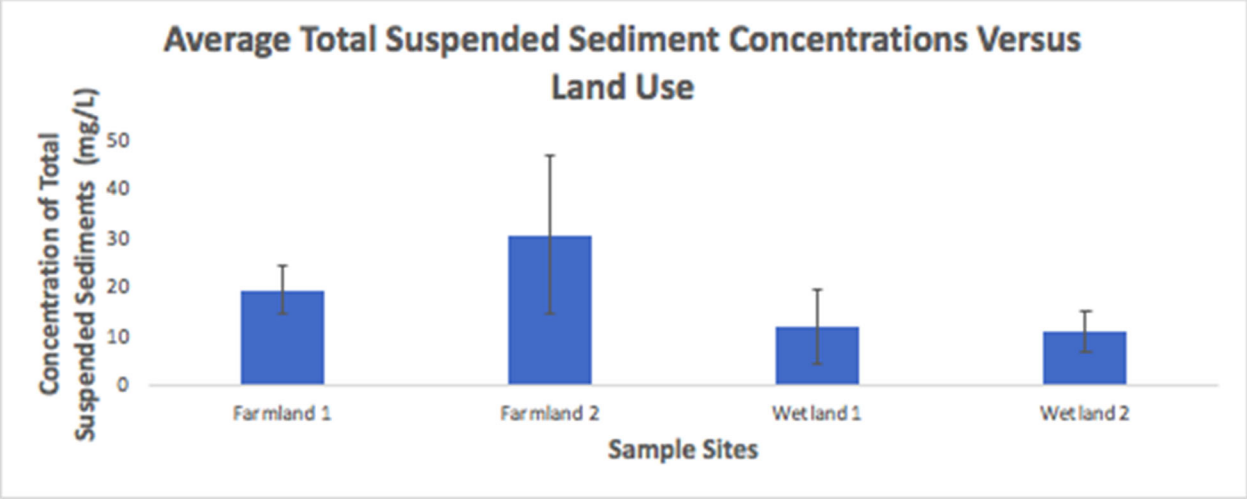

Figure 25. Average concentrations of Total Suspended Sediments at each sample site over the full course of data collection

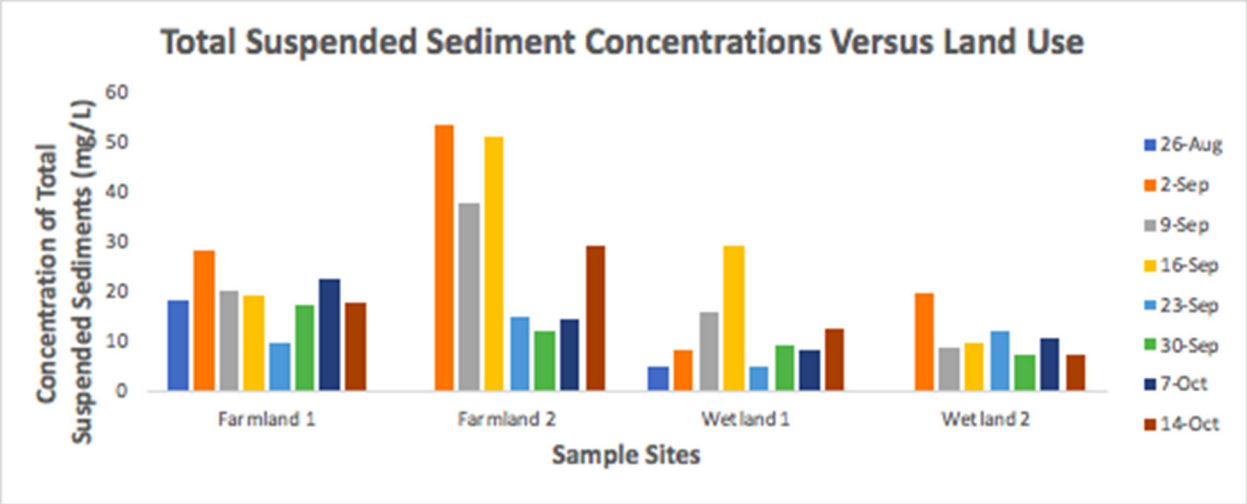

Figure 26. Total Suspended Sediment concentrations on each day of data collection

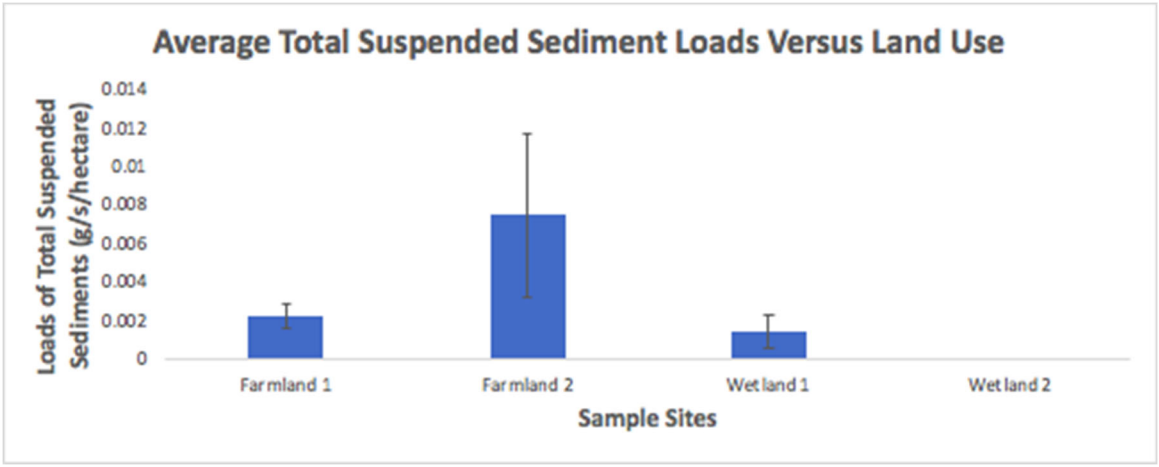

Figure 27. Average amounts of Total Suspended Sediment loads per sample site over the course of data collection.

3.4 Long Term Trends of Fecal Coliforms.

Ward Creek has shown a dramatic decrease in average fecal coliform concentrations after 2004 (Figure 28). All portions of both the North River and Ward Creek saw increases in fecal coliform concentrations during the time period from 2000 to 2004, largely due to the fact that this was the wettest 5 year period on record at the time since 1904 (Frankson et. al., 2019). Even discounting the average value from 2000-2004, Ward Creek still saw a decrease in average fecal coliform concentrations when compared to the 10 years from 1989 to 1999 (Figure 28). The North River showed a less consistent response to the restoration project (Figure 29).

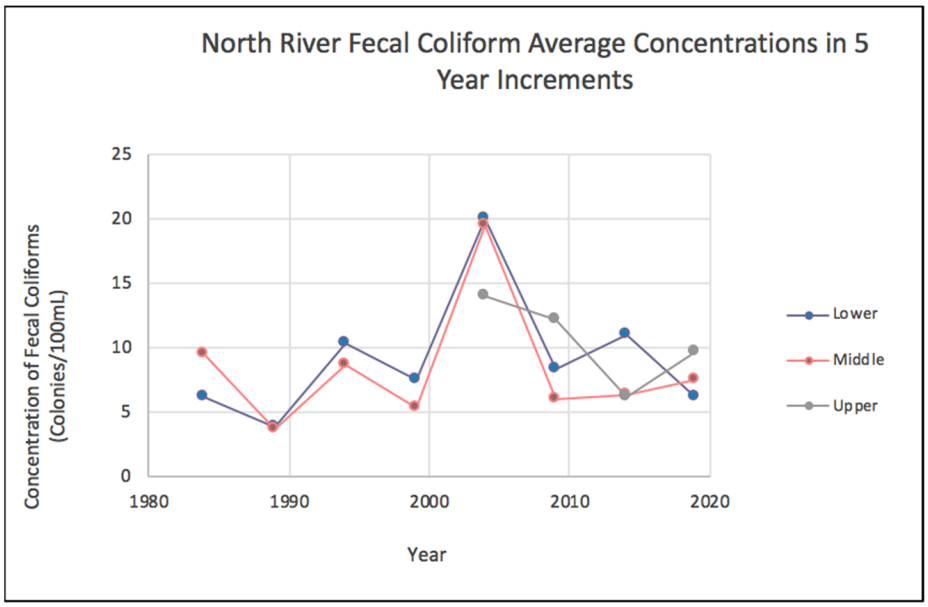

Figure 28. Average concentrations of fecal coliforms in the North River since 1983 in five year increments

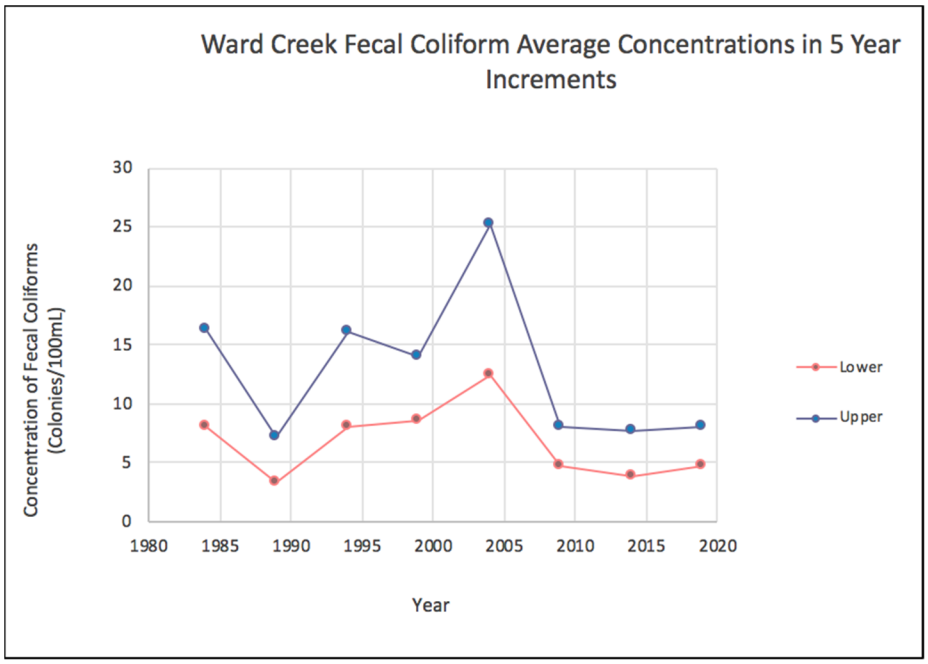

Figure 29. Average concentrations of fecal coliforms in Ward Creek since 1983 in five-year increments

4. DISCUSSION

4.1 Loads and Concentrations of Vibrio Species.

Based on our data, our hypothesis that the Farmland outfalls would have higher concentrations and loads of *Vibrio spp.* can be supported. For example, the second Farmland sample site consistently held the highest levels of *Vibrio spp.* loading while Wetland 1 held the lowest loads of *Vibrio spp.* (excluding Wetland 2, which did not experience noticeable water flow). However, this may be due to the manner in which the three sites interact with the larger estuary. The two Farmland sites receive agricultural runoff directly from the neighboring Open Grounds Farm and are directly connected to the tidally influenced North River and Ward Creek estuary systems. These two sources of input into the Farmland sample sites provide chances for material and bacteria to be deposited and ejected from the sites. The Wetland sample sites do not directly receive these inputs and are primarily filled by runoff from the restored wetland itself. The diminished tidal influence in the Wetland may also contribute to the low concentrations and loads of *Vibrio spp*. On the other hand, the strong tidal influence in the Farmland sites alters concentrations of *Vibrio spp.* by resuspending bacteria-laden sediments (Fries et. al., 2008). It is important to note that the incredibly high *Vibrio spp.* concentrations of September 9th were likely a result of a rainstorm the previous day washing nutrients into the farmland sites and providing ideal conditions for the *Vibrio spp.* present in the estuary to bloom. The fact that this rainfall did not cause similar blooms in the Wetland sites supports our hypothesis that *Vibrio spp.* does not proliferate well in the wetland sites. It is also important to note that *Vibrio spp.* are indigenous to estuarine systems and are not deposited into the outfalls by runoff.

4.2 Concentrations and Loads Fecal Indicator Bacteria and Total Suspended Sediments.

In terms of fecal indicator bacteria concentration values and standard deviation, the Wetland 2 site consistently showed the lowest values with the exception of total fecal coliforms (Figures 10, 13, 16). Wetland 1 also consistently showed lower concentrations than either of the Farmland sites. This observation supports the hypothesis that the North River Wetland Preserve is effective at reducing fecal indicator bacteria because the Wetland samples have lower concentrations of fecal indicator bacteria than the Farmland sites. Furthermore, Farmland 2 consistently showed the highest bacterial loads across all 3 types of fecal indicators analyzed. This is particularly noteworthy when considering the total coliform data because Farmland 2 did not boast the highest concentration of total coliforms. Farmland 1 held the highest total coliform concentration. The fact that Farmland 2 maintained the greatest load of total coliforms means that water flow was a far more significant factor in determining the magnitude of bacterial load. The Wetland sites showed higher concentrations of total coliforms than either *E. coli* or *Enterococci* (Figure 16). When one considers that total coliforms are the least specific of the fecal indicator bacteria we investigated, this could easily be the result of wildlife fecal droppings (Washington State Department of Health, 2020). Additionally, the turbidity caused by the rise and fall of the tides in the Farmland outfalls can cause resuspension of sediments, giving fecal indicator bacteria the chance to proliferate to concentrations that distort the original degree of fecal contamination (Fries et. al., 2008). This provides a potential mechanism for why the fecal indicator bacteria load of the Wetland sites is lower than the Farmland sites.

The Total Suspended Sediment load data largely resembles the fecal indicator bacteria loads, with the Wetland 1 site possessing the lowest load and standard deviation values in comparison with both of the Farmland sites (Figure 19). This provides additional support to our

hypothesis that the NRWP removes contaminants from runoff. This sediment removal likely contributes to the lower bacterial levels observed in the Wetland sites when compared to the Farmland sites because fecal indicator bacteria aggregate onto sediment particles (Fries et. al., 2010). Some research has shown that anywhere from 30-40% of *Vibrio* and fecal indicator bacteria attach themselves to suspended sediment particles, meaning that the wetland's reduction of TSS may inhibit the capacity of these bacteria to persist in the wetland's water bodies (Fries et. al., 2006).

4.3 Trends of Fecal Coliforms in North River and Ward Creek.

Since the upper portion of Ward Creek had the largest decrease in fecal coliform concentrations, we found that the restoration of NRWP was effective at improving water quality in that water body–supporting our hypothesis (Figure 29). The concentrations in this water body were rather variable in previous decades and, after 2004, these concentrations have fallen and remained consistently low when compared to pre-restoration annual concentrations. The North River itself, however, had variation both before and after the wetland's restoration, making it difficult to support our hypothesis regarding this water body. This may result from the visibly larger size of the North River when compared to Ward Creek (Figure 6). Another potential explanation is the presence of septic systems near the estuary. As coastal areas see rising levels of human development, the risk of septic contamination of coastal waters rises significantly (Parker et. al., 2010).

4.4 Confounding Variables.

This study faces several factors that may have influenced our results. One of the most noteworthy is that, as members of the Institute of Marine Sciences (IMS) Fall Field Site, we faced scheduling constraints that limited our ability to collect and process samples to once a week. The program specified Wednesdays as the primary day each week to work at NWRP, and we complied with this assigned schedule, collecting samples each Wednesday morning and returning to IMS to process the samples for the remainder of the day, analyzing the results of the fecal indicator bacteria and *Vibrio* samples the following day after overnight incubation. We were only able to observe bacterial concentrations in the mornings, while other researchers observing the impact of restored wetlands on fecal indicator bacteria loads did so in 24-hour surveys, enabling them to observe how bacterial loads rise and fall between day and night (Dorsey et. al., 2010).

Another potential distortion is the way that tidal variation alters the salinity of the water. During our study, we found that the Farmland outfalls had an average salinity of around 7 parts per thousand while the Wetland sites had an average salinity of approximately 1 part per thousand. This is relevant because the *Vibrio* species we investigated thrive in estuarine salinities, meaning that instances when the salinity was low may have led to abnormally low *Vibrio spp.* abundance, and vice versa. This constant change in salinity due to the tides likely caused the *Vibrio spp.* readings at the Farmland sites to vary widely when compared with the Wetland sites, which experience little tidal influence and have more consistent salinities. In addition, precipitation strongly influenced the bacterial concentrations of certain days. For instance, the farmland sites had high concentrations of *Vibrio spp.* on September 9th, a day after a fairly heavy amount of rainfall. As a result, the observed differences in *Vibrio spp.* concentrations and loads may not be due to the different sources of runoff but rather differences in salinity.

As discussed earlier, the NRWP provides habitat for a wide variety of wildlife by design. These organisms serve as an alternate source of fecal indicator bacteria, given that fecal coliforms

are not specific to human fecal waste or agricultural manure. Additionally, much of Carteret County North Carolina's population uses septic systems to dispose of their own sewage, serving as another potential source of fecal contamination. Finally, *Enterococci* are capable of surviving in estuarine systems independent of fecal matter, meaning this bacteria may not accurately reflect the presence of fecal contamination.

5. CONCLUSION

The restoration of North River farm into a wetland was initiated in the hopes of improving the environmental health of Morehead City, North Carolina. After conducting both observational and analytical studies, we determined that the restored wetland sites experienced lower concentrations along with lower loads of pathogenic bacteria, fecal indicator bacteria, and Total Suspended Sediments (TSS) than the farmland sites. The restoration project has been quite successful in improving water quality in both Ward Creek and the North River by reducing fecal coliform concentrations in areas nearest to the restored wetland.

CHAPTER 4: Nutrients

1. INTRODUCTION

The effectiveness of wetland restoration projects can be quantified in a variety of ways, and different wetland restoration projects can have varying degrees of success (Land 2016). Restoration effectiveness is important to quantify, considering these projects require a significant amount of investment (Steyer 2003). Nitrogen retention and removal is a coveted and important ecosystem service that wetlands provide, and is a driving motivation behind many wetland restoration projects. Wetlands remove nitrogen in a myriad of ways, two of which are nutrient uptake in plant biomass, and the microbial process of denitrification. In young constructed marshes, plant uptake is the dominant form of nitrogen retention, but as the marsh ages, denitrification plays a larger role (Etheridge et al, 2013; Craft et al. 2003). Nitrogen processing in wetlands normally follows the three-step microbial chain of ammonification, nitrification, then canonical denitrification. The speciation of N in the wetlands can indicate which process is most dominant. For this chain of bacteria-mediated reactions to be efficient, the wetland surface must be oxygenated so the nitrifying microbes can function to convert ammonium to nitrate (Maltais-Landry et al. 2009).

In this study, we investigated a wetland restoration project located in coastal North Carolina. The restored wetlands sit on top of the former North River Farms, and adjacent to a currently operational farm called Open Grounds. Open Grounds Farm is the largest row crop agricultural operation east of the Mississippi River. Formerly, much of their land was used as a pasture for livestock. They have since shifted to producing solely corn and soybean. Row crop agriculture requires lots of fertilizer; Open Grounds' previous application method was to apply nitrogen fertilizer evenly across the entire farm. They have been trying to reduce their fertilizer use, and their current method is to collect soil samples after harvesting their crops to establish nutrient levels within the soil and to determine the amount to apply the next season. These updated practices have reduced total nitrogen use by approximately 20%. Even so, excess nitrogen from fertilizer application still makes its way into the downstream estuaries of the North River. North Carolina estuaries tend to be nitrogen limited, so any fluxes of nitrogen from agricultural runoff can cause eutrophication and harm their health (Piehler et al. 2004).

The North Carolina Coastal Federation established the North River Wetland Preserve (NRWP) in 1999 to reduce the impacts of agricultural runoff and improve downstream water quality. The aim of this study was to quantitatively compare the ecosystem services provided by the restored wetlands to their pre-restoration state and to nearby natural wetlands, by examining nutrient concentrations and denitrification rates. We hypothesized that runoff from the restored wetlands would contain less nutrients than runoff from their pre-restoration state of farmland. We also hypothesized that natural wetlands would denitrify at higher rates than the restored wetlands and that the older restored wetlands would denitrify at higher rates than the younger ones. To test our hypotheses, we monitored nutrient concentrations in the water coming off of the restored wetlands and off of Open Grounds farm, which we used as a proxy for the pre-restoration state of the restored wetlands. We also performed a denitrification experiment using cores from the three restored wetlands, and from a nearby natural wetland in order to compare their denitrification rates.

2. METHODS

2.1 Nutrient Analysis

To monitor nutrient concentrations, water samples were taken from four locations. Two of the locations were farm outfalls (FR) that drained water from Open Grounds. The other two were wetland outfalls (WRR) which drained water from the restored wetlands. Two water samples were taken from each outfall every week. In addition to weekly water samples, three automated water sampling devices (ISCOs) were deployed at the FR1, FR2, and WRR1 outfalls from August 27th to October 14th. We were only able to acquire three ISCOs for this study, so we did not deploy one at the WRR2 outfall. The ISCOs were securely placed on the bank of each outfall, with the water inflow tube running down into the water and kept in place by a floatation device attached to a pole that was driven into the bottom of the channel. Each ISCO was also equipped with an acoustic flow meter and water level logger. These were attached to cinder blocks and placed at the bottom of the channel at the FR2 and WRR1 sites. At FR1, the flow meter was clamped to the side of a pipe. The acoustic sensors were originally programmed to record the water velocity and level every 30 minutes, but were reprogrammed to collect data every five minutes on September 23rd. The ISCOs were programmed to collect 500mL of water every eight hours. Each week, we replaced their water bottle carousels with clean bottles and took the full carousels to IMS for filtering.

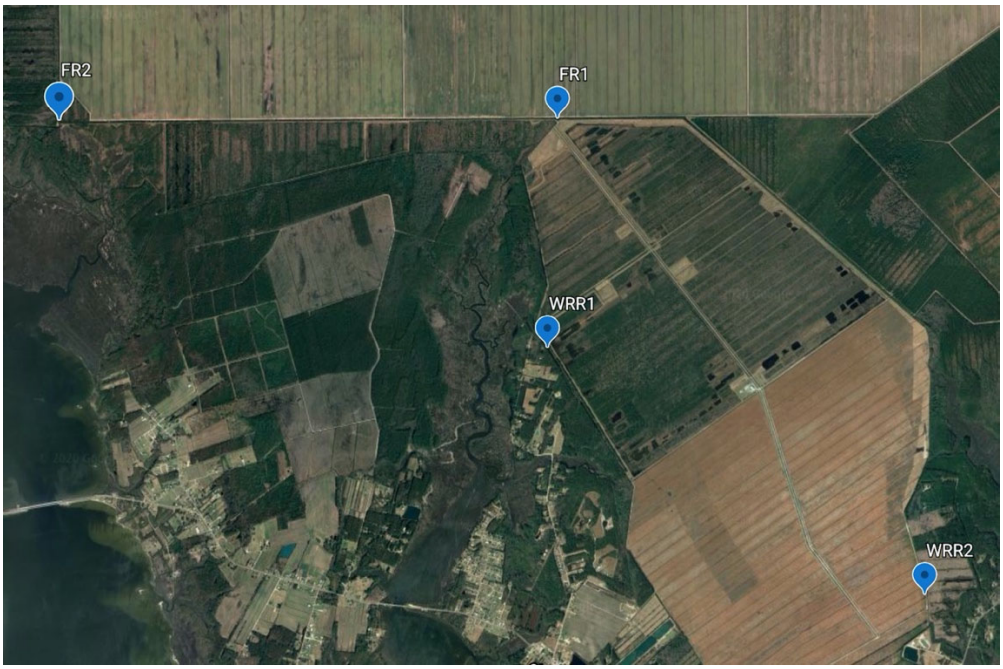

Figure 1. image depicting locations of two wetland outfall sites (WRR) and two farm outfall sites (FR).

In order to choose which water samples from the ISCOs to filter, we examined the water level and velocity data each week to detect rain events. We determined that upticks in velocity and water level indicated a rain event, and when we detected one we selected the water sample that most closely corresponded to the time when the rain event occurred.

The selected rain event samples were filtered in the Paerl lab at IMS along with the weekly water samples collected from the four outfall sites. 50 mL of each sample were filtered with a 25-micron fiberglass filter to remove any microorganisms and large sediment particles that would clog the lab instruments used for analysis, and frozen until they could be processed in a large batch. Filtered water samples were run through an auto-analyzer using standard methods to determine concentrations of $N\text{-}NO_x$, $N\text{-}NH_4$, and TDN following Smyth et al. (2013).

2.2 Denitrification Measurements

To measure denitrification, three cores were taken in transects from the 2007, 2013, and 2015 wetlands, as well as from a nearby natural wetland. Cores were taken by driving a plastic tube with an opening that was 6.4cm in diameter about 10cm into the sediment, filling the tube up with water, and plugging the top to create a seal which allowed the core to be pulled from the ground. The bottom of the core was then plugged to seal the sediment inside. In addition to the cores, several carboys were filled with water from Ward Creek to be used in the denitrification experiment. Cores were placed into a cooler for transport back to IMS.

Figure 2. Image depicting locations of cores taken from each wetland. Yellow markers correspond to 2007 wetland, orange markers correspond to 2015 wetland, green markers correspond to 2013 wetland, and blue markers correspond to natural wetland.

A continuous flow experiment was conducted to quantify the amount of nitrogen gas being produced by each core. The continuous flow experiment was conducted in a temperature controlled environmental chamber at 23°C. The environmental chamber was kept dark to prevent any photosynthetic microbial activity that would interfere with the gas exchanges being measured in the experiment. Before beginning the experiment, the top of each core was removed and they were submerged in oxygenated water collected from Ward Creek for a minimum of 12 hours to allow them to assimilate to the new conditions.

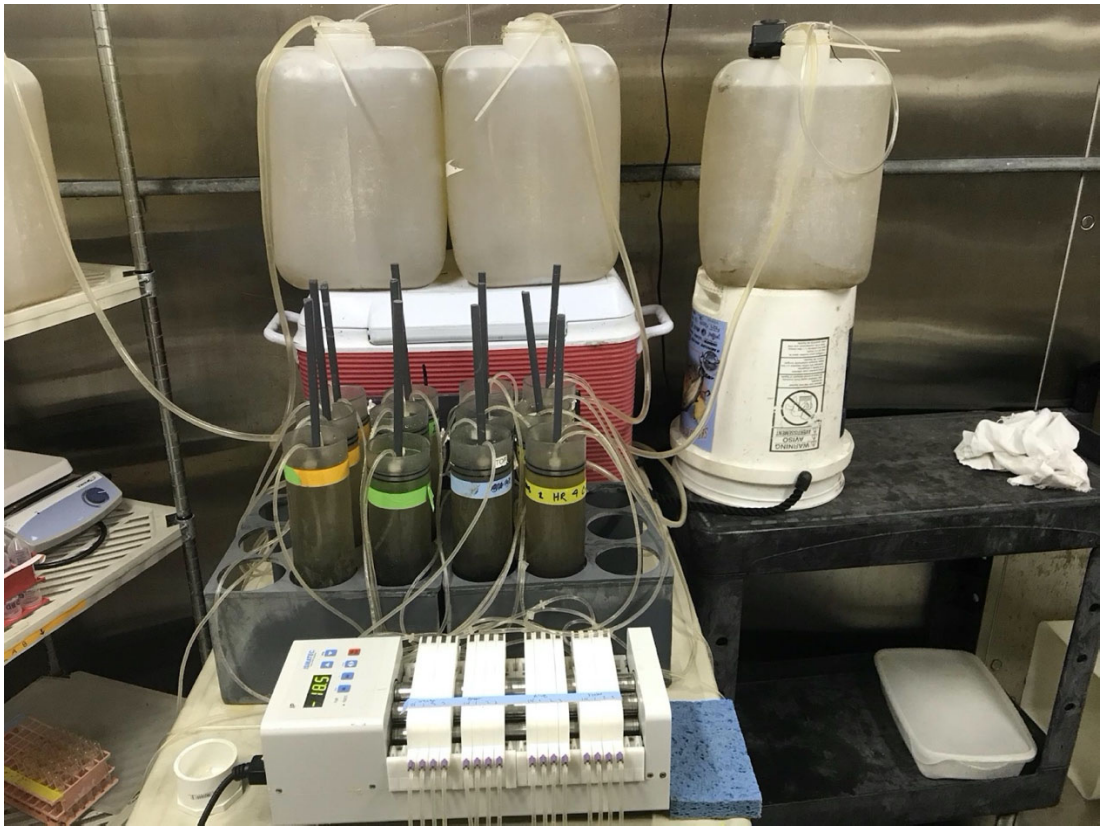

Figure 3. Image depicting setup of continuous flow denitrification experiment in environmental chamber.

After the 12-hour period, each core was capped with a plexiglass topper that contained two ports plumbed with Tygon tubing for inflow and outflow water. The inflow tubes were hooked up to a reservoir of oxygenated Ward Creek water which was pumped over the cores using a peristaltic pump at a rate of one mL per minute. Samples of inflow water from the reservoir and outflow water from each core were collected in 5mL glass test tubes and capped. The samples were then analyzed using a Membrane Inlet Mass Spectrometer (MIMS) to determine the concentrations of dissolved N_2, O_2, and Ar in the inflow and outflow (Poe et al. 2003). Water samples were run through the MIMS five different times, with two runs occurring on September 29th, two on September 30th, and a final one on October 1st. In between the second and third run, nitrate was added to the reservoir water to increase its concentration to 50 micromolar to simulate a flux of nitrogen coming off of a farm during a storm. Samples of reservoir water were collected immediately before and after the spike, filtered, and measured by the auto-analyzer to find nutrient concentrations.

2.3 Data Analysis

To find dissolved inorganic nitrogen (DIN) of each sample, concentration values for N-NO_x and NH_4 were summed. To find dissolved organic nitrogen, DIN concentrations were subtracted from TDN concentrations reported by the auto-analyzer. To determine when rain events occurred, we used the US1NCCR0009 station in NOAA's record of climatological observations to see if any rainfall was recorded on each of our sample days, and looked for any increases in water level and velocity from the data recorded by the ISCOs. To calculate the average wet and dry concentrations for each site, we used NOAA's record of climatological observations, and included every sample collected on a day when rainfall was recorded in the average for wet days. The dry day average included concentrations for every sample day where no rainfall was recorded. Average flow was calculated by averaging all of the velocities at each site together and multiplying by the cross-sectional area of each outfall. To find loads, we multiplied the average concentrations of DIN, DON, and TDN by the average flow at each outfall.

To calculate the denitrification rates of each core, we multiplied the product of the ratio of N_2:Ar from each core and the micromoles of Ar that there would be under ideal conditions by two to get micromoles of N. We then subtracted the micromoles of N in the core inflow water from the micromoles of N in the water leaving the cores. We multiplied the difference by the ratio of the pump rate of the water to the area of the cores to end up with µmol N/m^2/hr. To correct the denitrification rates for inundation, we found the percent inundation values for the exact locations of each core, and averaged them together for each wetland. We then multiplied the average percent inundation by the denitrification rate for each wetland.

3. RESULTS

Inorganic and organic forms of dissolved nitrogen varied greatly at all sample sites throughout the study period. Dissolved inorganic nitrogen (DIN) ranged from 49µg/L to 612µg/L at FR1, from 30 to 310µg/L at FR2, from 9 to 78µg/L at WRR1, and from 23 to 228µg/L at WRR2 (Figure 4). Dissolved organic nitrogen (DON) ranged from 116 to 728µg/L at FR1, from 142 to 623µg/L at FR2, from 103 to 411µg/L at WRR1, and from 831 to 1752µg/L at WRR2 (Figure 5). On average, the farm outfalls had higher concentrations of DIN than the wetland outfalls, while the wetland outfalls had higher concentrations of DON than the farmland outfalls (Appendix: Figures 4, 5).

Dissolved nitrogen concentrations at the farm and wetland outfalls seemed to have different relationships with rainfall events. At FR1, TDN concentrations were around 500µg/L 24 hours before the rain event detected on September 17th. TDN then fell to about 200µg/L at 7:30am on the 17th, then shot up to about 1100µg/L around 4:00pm (Appendix: Figure 6). WR2 followed the same pattern, with TDN the day before the rain event reaching 344µg/L, falling to 161µg/L at 7:30am, then climbing to 401µg/L around 4:00pm (Appendix: Figure 7). FR2 displayed a different pattern, with TDN concentrations starting at 119µg/L the day before the rain, then reaching a peak at 933µg/L before falling to 590µg/L (Appendix: Figure 8). At FR1 and FR2, both DON and DIN fluctuated throughout the course of the rain event (Appendix: Figures 4, 5). At WRR1, DON fluctuated greatly, but DIN stayed at about the same concentrations throughout the event (Appendix: Figure 6). We do not have concentration data for WRR2 during the rain event, but TDN was 1885 µg/L 24 hours before the rain event, with DON sitting at 1657 µg/L (Appendix: Figure 9). The discrepancy in N concentrations between the two

farm outfalls may be related to the stage of the tidal cycle at the time of sampling, as the tide was staggered between the sites. The highest concentrations of TDN seen during the rain event at each respective site were from the samples that were collected at low tide. This is likely because at low tide, the water in the outfalls is mostly made up of runoff from the farm and wetland, as opposed to high tide when much of the water is from downstream (Appendix: Figure 10).

At FR1 and FR2, the average DIN concentrations were 52% and 38% higher respectively on rainy days than on dry days where no precipitation was measured. DIN concentrations at WRR1 and WRR2 were 28% and 11% lower respectively on rainy days than dry days (Appendix: Figure 11).

Water level at both farm outfalls and WRR1 had significant tidal influences. FR1 experienced the greatest fluctuation in water level, while WRR1 experienced the least amount of change (Appendix: Figure 10). WRR2 did not have a detectable tidal signal. FR2 had the highest average flow rate at 9.89ft^3/s, while FR1 and WRR1 had significantly lower average flow rates at 3.90ft^3/s and 1.08ft^3/s respectively (Appendix: Table 1). We never detected flow at WRR2. These flow rates correlate to the loads of dissolved nitrogen at each site. The loads of TDN coming off of FR1, FR2, and WRR1 were 2.2, 3.8, and 1.0 kg/ha/year respectively. DON made up the majority of total dissolved nitrogen at all outfall sites. Loads of both DIN and DON coming off of the two farm outfalls far exceeded loads coming off of the wetland outfalls (Appendix: Figure 12).

Denitrification rates were similarly high across all of the wetland sites. During the first two runs of inflow and outflow through the MIMS, denitrification rates ranged from 704 to 870 $\mu mol/m^2/hr$ (Appendix: Figure 13). Before the third run of the experiment, the nitrate concentration in the water flowing over the cores was increased to 50µM, or around 640 µg/L, which was significantly higher than most recorded concentrations of NO_x in our water samples (Appendix: Table 2). On two occasions, once during a rain event and once during a falling tide, nitrate concentrations at FR1 reached levels in the 500µg/L range, but that was not a regular occurrence. After the addition of nitrate to the inflow water, the denitrification rates of the cores from the 2013, 2015, and natural marshes dropped to levels ranging from 72$\mu mol/m^2/hr$ in the 2013 cores to 112 $\mu mol/m^2/hr$ in the natural cores. The rates of the 2007 marsh cores also decreased from 850$\mu mol/m^2/hr$ to 584$\mu mol/m^2/hr$. By the end of the experiment, the denitrification rates of the 2015 cores had only increased to 129 $\mu mol/m^2/hr$. The denitrification rates of the 2013, 2007, and natural marsh cores on the 5th run were 335, 519, and 479$\mu mol/m^2/hr$ respectively (Appendix: Figure 13). The average dissolved oxygen concentration measured by the MIMS in the core inflow water before the nitrate addition was 7 mg/L, and after the addition it was 3 mg/L (Appendix: Figure 14).

After correcting the denitrification rates to reflect percent inundation (Appendix: Table 3), the 2013 and natural marshes displayed the highest rates of denitrification, at 593 and 546 $\mu mol/m^2/hr$ respectively. The 2007 and 2015 marshes showed lower rates of denitrification 339 and 180 $\mu mol/m^2/hr$ respectively. Though lower than the uncorrected inundation values, all denitrification rates still far exceeded the regional average denitrification rate of 37$\mu mol/m^2/hr$, which was retrieved from literature (Smyth et al. 2013; Appendix: Figure 15).

4. DISCUSSION

The objectives of our study were to quantify the concentrations of nutrients in the runoff from Open Grounds farm and from the restored wetlands at the NRWP to be able to compare the

restored wetlands to their previous state. We also aimed to determine how rates of denitrification change as wetlands age. Our results demonstrate that DIN concentrations are, on average, higher coming off of farmland than wetland. When rain events occur, there is no large release of inorganic nitrogen from the wetlands, but there is from the farmland. The farmland also releases more organic and inorganic nitrogen per hectare than the restored wetlands. These results support our first hypothesis that farm runoff would be more nutrient rich than wetland runoff, and demonstrate that the restoration of the wetlands has decreased the loads of nitrogen coming off of the former North River farms.

The WR2 outfall did have the highest concentrations of DON, but this does not necessarily negate our conclusions. The high DON concentrations are likely due to the presence of a water control structure at the site, which inhibited water flow and may have allowed DON to accumulate. Our description of nitrogen loads and concentrations is only relative to the outfalls we studied at the NRWP. TDN loads across all outfalls that we measured are low compared to large agricultural drainage basins in the states of Minnesota, Iowa, Illinois, Indiana, and Ohio, where loads range from 8 to 31 kg/ha/yr (Goolsby et al. 2000). A 2011 study of the hardwood wetlands at the NRWP found that on average they exported 12kg/ha/year of TDN, so the restored wetland marshes that we studied have low loads even for the wetland preserve (Burchell et al. 2011).

While the results of our nutrient monitoring methodology supported our hypothesis, there are gaps in the data that could possibly be confounding. We were only capable of collecting water samples from each site on allocated workdays, which were once or twice a week, and we were only able to deploy three ISCO water samplers, so we could not collect regular water samples from WRR2. Etheridge et al.'s (2014) study of similar constructed wetland sites nearby found that nutrient concentrations were influenced by the stage of the tidal cycle when the weekly water samples were grabbed, and we did not collect samples at the same point in the tidal cycle each week. Etheridge et al. (2014) also found that when measuring nitrogen fluxes coming off the restored wetlands, the difference of calculated final mass between a two-hour sampling frequency and 15 minute sampling frequency could be over 100% (Etheridge et al. 2014). Since our sampling frequency was once a week, we likely missed data points that would have better informed our conclusions. We were also working with low resolution data regarding when rainfall events occurred. It was difficult to determine when it rained from velocity and water level data alone, because of the strong tidal signal at the three sites with ISCOs. We determined wet and dry days using NOAA's records of climatological observations, which only states inches of rainfall per day, meaning we could have taken water samples before the rain event occurred and still counted them as wet days.

The results of our denitrification experiment demonstrate that all of the restored wetlands are capable of high rates of denitrification, but the 2013 and natural marshes had the highest rates of denitrification because of their higher inundation frequency. This goes against our second and third hypotheses that the natural wetland would denitrify at higher rates than the restored wetlands, and that the older restored wetlands would denitrify at higher rates than the younger ones. Instead, we found that all wetlands have similar denitrification rates when inundated, which leaves the time a wetland spends inundated as the deciding factor in how much denitrification can occur. Poe et al.'s (2003) study of a constructed wetland on Open Grounds farm found an average potential denitrification rate of 179 $\mu mol/m^2/hr$, which is lower than the average denitrification rate of all of our cores, 801 $\mu mol/m^2/hr$. This study monitored the denitrification rates over a longer period than ours, and found a high of 657 $\mu mol/m^2/hr$ in the

summer and a low of 50 µmol/m²/hr in the fall, which was when our denitrification experiment was conducted.

Poe et al. (2003) also found that denitrification rates increased when nitrate concentrations in the water increased. After a fertilizer spill upstream that resulted in a nitrate concentration of 21000µg/L, their denitrification rates increased to 1400µmol/m²/hr (Poe et al. 2003). When we increased the nitrate concentration in the inflow water of our denitrification experiment, our denitrification rates decreased to around 100 µmol/m²/hr. It is possible that this is due to a 56% decrease in the dissolved oxygen concentration of the inflow water from before to after the addition of nitrate (Appendix: Figure 15). In hypoxic conditions, dissimilatory nitrate reduction to ammonium (DNRA) tends to dominate over denitrification processes (Jäntti and Hietanen, 2012). Instead of converting nitrates to N_2 gas, nitrate would be converted to ammonium and remain in the system, leading to a drop in denitrification rates. We do not have nutrient concentration data for the inflow and outflow water apart from immediately before and after the nitrate addition, so we cannot examine this hypothesis by determining whether levels of ammonium increased after the denitrification rates dropped.

5. CONCLUSION

This study provides evidence that the restored wetlands at the NRWP release less nitrogen into the downstream estuarine system than their pre-restoration state of row crop agriculture. Additionally, the denitrification rates of the restored wetlands were similarly high to the rate of the natural wetland when inundated with water. We concluded that denitrification rate is more dependent on the amount of time a wetland spends inundated with water than its age. These conclusions can inform the implementation of future wetland restoration projects. Future designs should aim to maximize the inundation frequency of the wetland by considering the elevation of the land and the tidal range of the water channels that connect the wetlands to downstream estuaries.

CHAPTER 5: Habitat Quality

1. INTRODUCTION

A significant goal of any wetland restoration project is to create and maintain a habitat which can support a variety of flora and fauna. Wetlands today occupy only 9% of the surface of the Earth's landmasses, yet provide biodiversity support that far exceeds what that number would suggest (Zedler and Kercher 2005). Historically wetlands have been degraded by human activity in a multitude of ways, primarily from wetland filling, and this degradation can inflict a noticeable decline in floral and faunal biodiversity within them (Lougheed et al. 2008). In this study, we examined the biodiversity of plant and invertebrate species at three different restored wetlands on the North Carolina Coastal Federation's (NCCF) North River Wetlands Preserve, each restored at different times (2007, 2013, and 2015).

Quantifying and comparing biodiversity across separate locations is a task that has been undertaken by numerous studies. Bioindicators are used to measure the health of an ecosystem, and invertebrates have been widely regarded as a good bioindicator for measuring many aspects of an ecosystem, including biodiversity (Schulze et al. 2004; Coscaron et al. 2009; Mauricio da Rocha et al. 2010). Compared to larger animals, insects and arachnids typically occupy small areas of land in their lifetime and can more easily be compared between restored wetland sites that are separated by relatively short distances. Vegetation also shares this characteristic with invertebrates. Due to their position at the base of the trophic level pyramid in most ecosystems, plant and invertebrate species both have an impact on what higher trophic level fauna is present and in what quantity (Barnes et al. 1995); greater biodiversity and abundance of these trophic-base organisms supports a larger number of organisms higher up (Barnes et al. 1995).

This study compared biodiversity of invertebrates and plant species between the three restored wetlands of varying age and design. Both vegetation count and insect diversity were utilized by our study as indicators of biodiversity. This study aims to determine the community composition of wetland and whether these differences are driven by age of the wetland, by plant communities, or both. We hypothesized that the older wetlands would feature the most abundance and biodiversity of insects and arachnids and vegetation. We also hypothesized there would be differences in invertebrate community composition based on the vegetation makeup of each restored wetland. To test these hypotheses, we compared the abundance and biodiversity between each wetland and used this information to determine both whether a restored wetland's age affects how biodiverse it is and how the plant communities in each wetland influenced the insect and arachnid biodiversity of these communities. Results may be used to determine which method of planting a restored wetland produces the highest biodiversity of plant and invertebrate species, and what duration of time it will take these projects to achieve peak biodiversity and the resultant ecosystem services.

2. METHODS

We selected three restored wetland sites of different ages and designs. The 2007 and 2015 wetlands were both engineered with a deep channel running through the middle of them and higher ground around it, whereas the 2013 wetland was dug out into a bowl shape, featuring very low elevation ground throughout and a less defined channel (Figure 1). We divided each wetland into strata based on the dominant habitat type observed from the ground and ecotones

observed in drone photography primarily based on vegetation. The 2015 and 2007 sites were split into a near creek (NC) and juncus (J) dominated habitat type while the 2013 site was considered all one hodgepodge (HP) strata.

Figure 1. Aerial photography of wetland sites with habitat boundaries added. From left: 2007 site, 2013 site, 2015 site. In 2007 and 2015 the lighter shaded area was the NC habitat type, darker shaded area was J. Photos provided by Hydrology group, taken two weeks before sampling began.

Each habitat type was sampled using active and passive methods at 2 randomly chosen locations. The 2013 site was considered a homogeneous habitat and was randomly sampled 4 times to match the total samples per wetland of the other two sites. Each site was sampled once weekly, for five weeks. Sampling plots were chosen by overlaying a 2 x 2 m plot with UTM coordinates over a map of the site and blindly pointing to a location until two points were chosen in each strata. The coordinates at that location were identified using the overlay and matched as closely as possible in the field using the Coordinates app (Mapnitude, 2020).

The invertebrate sampling methods we used were chosen to sample the widest range of species while limiting our collection to those that lived mainly within the marsh being sampled. Yellow pan traps were used primarily to capture insects living among the marsh grasses or close to the ground and have been used in many other insect studies (Musetti 2019; Figure 2). Sweep sampling was performed to capture species living in the mid to upper level of marsh grass (Rudd and Jensen 1977). Light trap sampling was performed to capture flying insects and those attracted to light more so than the yellow color of the pan traps (Sheikh 2016; Cadmus et al. 2016; Figure 3). Due to technical malfunctions that prevented even sampling of sites in the time provided, light trap data was not used in this study.

Figure 2. Example of a yellow pan trap

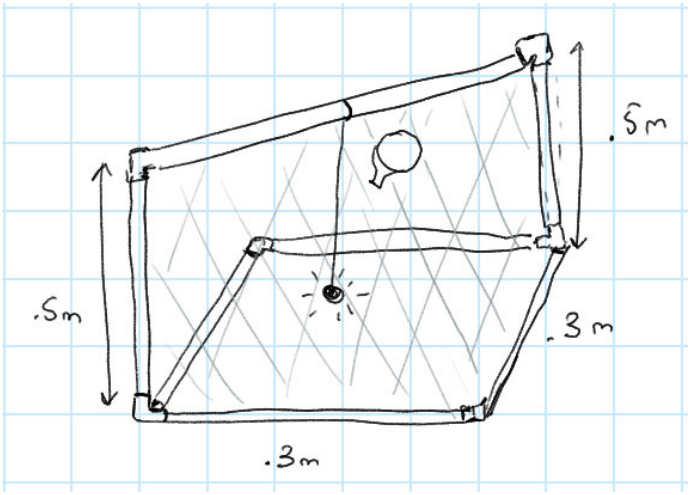

Figure 3. Example of a light trap design

At each sampling point, one yellow pan trap was deployed for 24 hours. After the 24 hours, the pans were collected and invertebrates sieved out and placed into a plastic baggie labeled with the trap letter, strata, and date. Each baggie contained all invertebrates collected in the same habitat type on that day and was then placed in a freezer until counted and identified. In the 2013 site, the four sampling points were arbitrarily split into two baggies to keep per effort measures consistent. Sweep sampling was performed once per habitat type (twice per marsh) in the 2007 and 2015 marsh and once per two sampling points in the 2013 marsh while collecting pan traps. This sampling method involved using a bug net to sweep through the surrounding marsh grasses in a roughly 2 m diameter circle around the spot where the yellow pan trap was placed (Rudd and Jensen 1977). Any invertebrates caught in the net were then transferred to a jar with acetone then placed in a baggie to be frozen. The same net was used for each sweep survey to maintain consistency. One light trap was deployed in the center of each marsh, or as close as possible, at the same time as the yellow pan traps and collected after 24 hours using the same method of collecting the bugs with acetone and freezing them in a baggie (Sheikh 2016; Cadmus et al. 2016).

Vegetation sampling was also performed on trap collection days. A ¼-meter by ¼-meter quadrat was placed on an area of representative vegetation within the same 2x2 m randomly chosen sites as the yellow pan traps. A shoot count of all identifiable species was then performed in one quarter of the quadrat. Samples of unidentified species were collected and taken back to the lab for identification.

Invertebrate identification was performed a week after collection. We attempted to identify each specimen down to the order using a light microscope and identification resources (NC State, 2015; Espace, 2019; UC Dept. of Ag, 2020). We also grouped each specimen into morphospecies based on their visual characteristics. Those invertebrates that appeared very similar to each other were considered the same morphospecies and we documented each new morphospecies with an image so that newly collected samples could be compared to older samples.

3. RESULTS

Comparing across the chronosequence, the 2007 pan traps had the greatest number of orders, while 2013 had the greatest abundance (Figure 4). In sweep data, the greatest number of orders was found in the 2007 site, and 2015 had the greatest abundance (Figure 5). The 2007

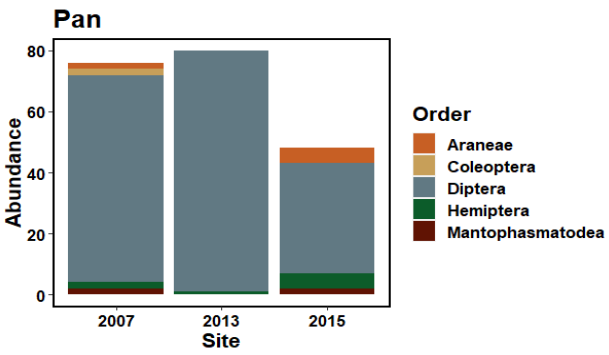

Figure 4. Our yellow pan trap data showed invertebrate abundances are much higher in å 2007 and 2013 site than in 2015. Diptera dominated in all sites, although 2007 showed the most variety in orders and 2015 had the greatest abundance of non-Diptera orders.

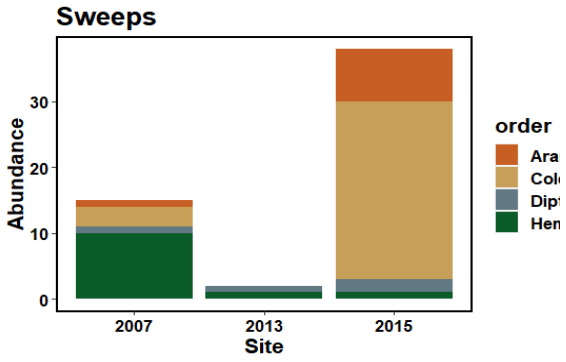

Figure 5. Our sweep data reflected higher diversity than the pan traps, with significantly higher abundance in 2015 than in the other wetland restoration sites. 2013 in particular featured very little sweep captures of insects.

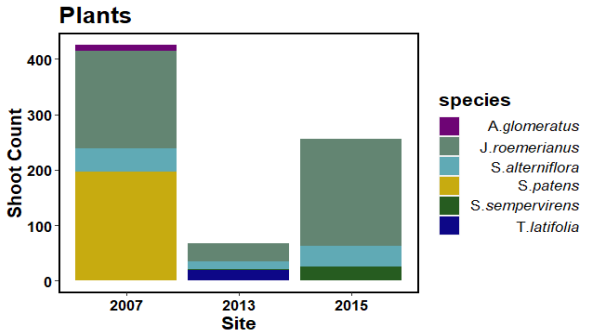

Figure 6. 2007 featured the highest abundance and diversity of plant species based on shoot count. 2013 in particular featured a much lower stem density than the other sites.

marsh had the highest shoot count and species present with 688 shoots per meter squared and four dominant species (Figure 6). Pan traps had the highest average catch abundance in the 2013 marsh, and the lowest in the 2015 marsh, however abundance did not significantly differ between the three marshes (Table 1). The highest average abundance in sweep surveys was in the 2015 marsh (8.2 invertebrates per sampling effort), the lowest was in 2013 (0.5 invertebrates per sampling effort). The mean abundance in each marsh was significantly different from the other marshes. The highest average shoot density was 688 shoots/m^2 in the 2007 marsh, and lowest was 134.4 shoots/m^2 in the 2013 marsh. Average shoot density was significantly different in each marsh (Table 1).

Table 1. Average Abundance across Marshes

Data type	2007	2013	2015
Abundance Average Pans	15.6 (10.7-20.5) *	16 (11.3-20.7)	10 (6.6-13.4)
Abundance Average Sweep	2.8 (0.9-3.9) **	0.5 (.28-.72) **	8.2 (4.8-11.6) **
Average Shoot per m^2	688 (546.4-829.6) **	134.4 (88.8- 180) **	443.2 (371.2-515.2) **

* Standard error
** Significantly different values

The highest average Shannon-Weiner biodiversity index for pan traps was found in the 2015 marsh and the lowest in the 2013 marsh (Table 2). Biodiversity was significantly lower in the 2013 marsh than the other two sites. The greatest average biodiversity caught in sweep surveys, 0.909, was in the 2015 site, while the lowest was in 2013 which showed zero biodiversity, a significantly different value from the other sites. The highest average plant biodiversity was in the 2007 marsh and the lowest in the 2013 marsh however the means were not significantly different (Table 2). Rarefaction plots for both pan traps and sweep surveys indicate more sampling was needed to capture total biodiversity (Figures 7 and8).

Table 2. Average Shannon-Weiner Diversity Index across Sites

Data type	2007	2013	2015
Average SW Pans	0.562 (0.386-0.737) *	0.049 (0-0.098) **	0.654 (0.255-0.860)
Average SW Sweep	0.647 (0.473-0.821)	0	0.909 (0.815-1.0020
Average SW Vegetation	.73 (.63-.82)	.58 (.47-.68)	.72 (.57-.87)

* Standard error
** Significantly different values

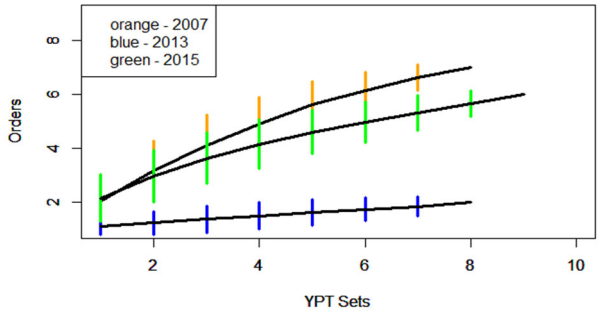

Figure 7. This plot shows how the number of invertebrate orders observed increased as the number of yellow pan trap samples increased. The plot shows that orders found at all restoration sites types were continuing to increase with sampling so the total species richness cannot be predicted. The vertical color bars indicate the confidence interval of orders at each sampling effort. From the observed trends we expect the 2007 site to show the highest total order richness and the 2013 site the lowest with this sampling mechanism.

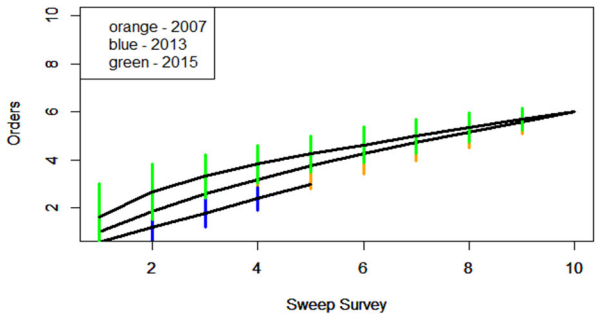

Figure 8. This plot shows how the number of invertebrate orders observed increased as the number of sweep surveys increased. The plot shows that orders found in all habitat types were continuing to increase with sampling so the total species richness cannot be predicted. The vertical color bars indicate the confidence interval of orders at each sampling effort. From the observed trends we expect the 2007 and 2015 site to have similar order richness, while the 2013 site we expect to have the lowest order richness.

Analysis of the relationship between Shannon-Weiner diversity of plants and invertebrates showed a weak positive relationship in the 2015 marsh in both pan traps ($R^2 = 0.11$, $p = 0.39$) and sweep sampling ($R^2 = .20$, $p=.19$). The 2013 marsh showed no correlation ($R^2 = 0.00$, $p = 0.89$). In the 2007 marsh correlation depended on the sampling method with pan traps showing a weak negative relationship ($R^2 = 0.03$, $p = 0.66$) and a weak positive relationship in sweep data ($R^2 =.07$, $p=.45$) (Figures 9 and 10).

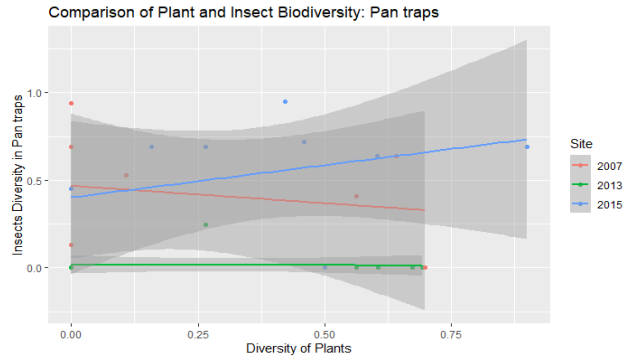

Figure 9. Comparing Shannon-Weiner biodiversity values for plant and Invertebrate communities showed different relationships at each site. A weak positive relationship was observed in the 2015 marsh and weak negative relationship in the 2013 marsh. The gray shaded areas show 95% confidence intervals for the linear model. Only one insect order was ever found in the 2013 marsh and therefore no relationship was found.

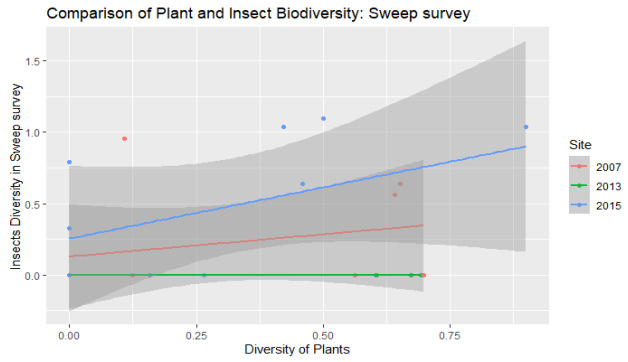

Figure 10. Shannon-Weiner diversity index values for plants and invertebrates caught in sweep surveys were compared for each site. There are weak positive relationships between plant and invertebrate diversity in 2007 and 2015, and no relationship in 2013. The gray shaded areas show 95% confidence intervals for the linear model. This demonstrates no strong relationship between vegetation and invertebrate diversity in these sites.

Comparing across habitat types, the highest total abundance caught in pan traps was in the HP habitat, which is entirely in the 2013 marsh, while the NC habitat had the most orders (Figure 11). Sweep surveys found the NC strata had the greatest number of orders and abundance (Figure 12).

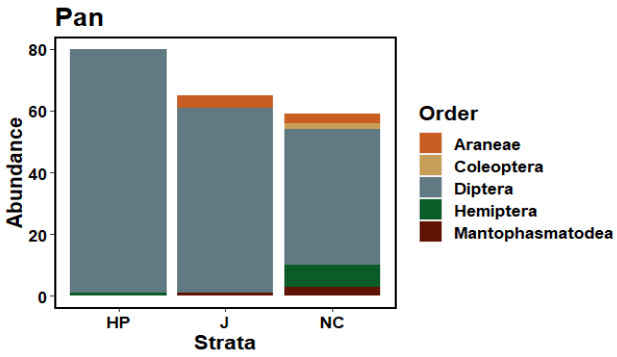

Figure 11. Pan trap order counts based on habitat type (strata). Hodgepodge had the highest abundance out of the three, but also the least diversity. Near Creek had the most diversity yet the least total abundance, but only slightly less than Juncus.

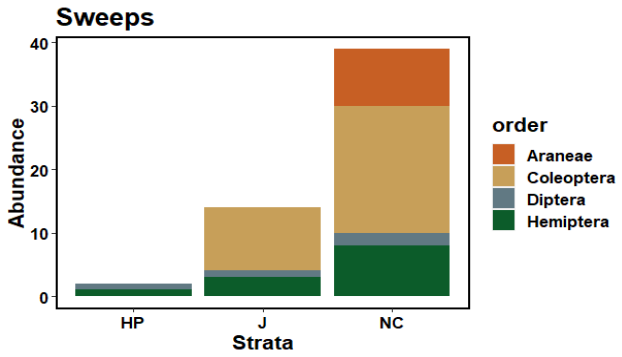

Figure 12. Sweep collection order counts based on habitat type (strata). Hodgepodge had very low abundance. Juncus had higher abundance and diversity, but not as high as the Near Creek strata.

The NC strata had the most diverse vegetation species and the greatest abundance of shoots (Figure 13). Average abundances of invertebrates in pan traps were highest in HP, where we found 16 invertebrates per sampling effort, and lowest in NC, although these were not significantly different. The highest sweep survey average was 7.17 individuals per sampling effort in NC, which was significantly higher than the other two marshes, and the lowest was 0.50 individuals per sampling effort in HP (Table 3).

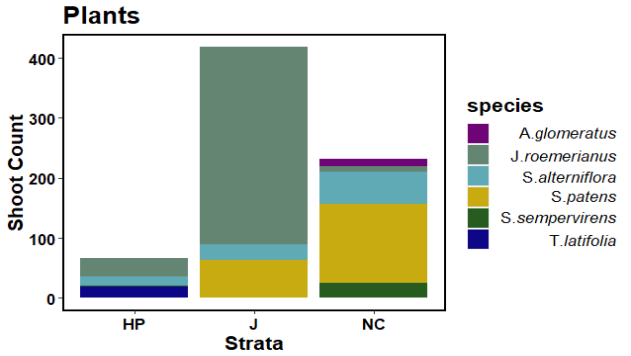

Figure 13. Plant survey species count based on habitat type (strata). Hodgepodge had the lowest abundance, while Juncus had the highest abundance and comparable diversity to HP. Near Creek had the highest diversity and an abundance between the other strata.

Table 3. Average Abundance by Habitat Type

Data Type	Near Creek	Juncus	Hodgepodge
Abundance Average Pans	12.2 (9.31-15.09)***	13.4 (7.48-19.32) **	16 (13.58-18.42) **
Abundance Average Sweep	7.17 (6.95-7.39) **	2.50 (0.87-4.13) **	0.50 (0-2.39) **
Average Shoot per m^2	387.2 (236-538.4) **	744 (654.4-833.6) **	134.4(88.8-180) **

* Standard error
** Significantly different values

The highest Shannon-Weiner diversity index averages across habitat types in pan traps was found in NC habitat (0.759) and the lowest in HP habitat (0.033). Each marsh had a significantly different mean from the other marshes. Sweep data showed the same pattern. Biodiversity of vegetation was greatest in the NC habitat, with an index of 0.75, which was significantly different from the other two marshes, and lowest in the J strata which had an index of 0.44 (Table 4). Analysis of rarefaction plots show more sampling was needed to get a complete total of orders found in each habitat, however NC appears to be trending towards having the highest number of orders in both sampling methods (Figures 14 and 15).

Table 4. Average Shannon-Weiner Diversity by Habitat Type

Data type	Near Creek	Juncus	Hodgepodge
Average SW Pans	0.759 (0.623-0.894) **	0.409 (0.269-0.549) **	0.033 (0.004-0.063) **
Average SW Sweep	1.45 (1.33-1.58) **	0.178 (0.063-0.292) **	0 **
Average SW Vegetation	0.75 (.71-.79) **	0.44 (0.26-0.62)))	0.58 (0.47-0.69) **

* Standard error
** Significantly different values

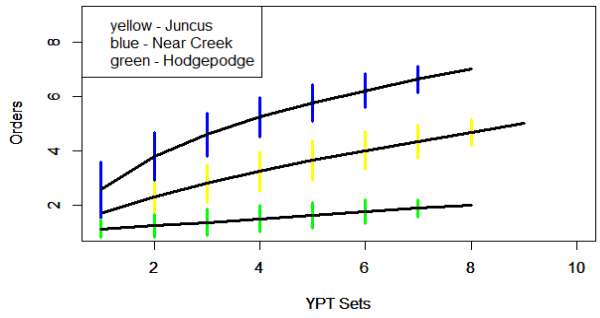

Figure 14. This plot shows how the number of invertebrate orders observed increased as the number of yellow pan trap samples increased. The plot shows that orders found in all habitat types were continuing to increase with sampling so the total species richness cannot be predicted. The vertical ranges of values indicate standard error of orders at each sampling effort. From the observed trends we expect the Near Creek strata to show the highest total order richness and hodgepodge the lowest with this sampling mechanism.

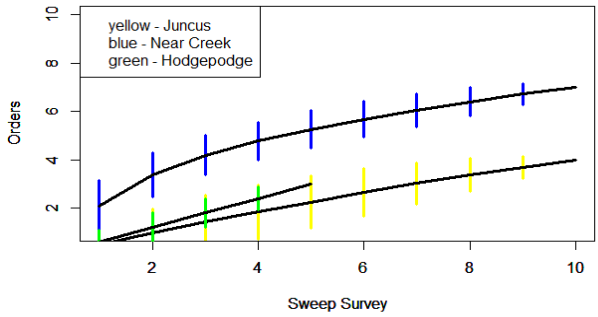

Figure 15. This plot shows how the number of invertebrate orders observed increased as the number of sweep surveys increased. The vertical ranges of values indicate standard error of orders at each sampling effort. The Juncus and Near Creek plots appear to be leveling out to an asymptote, however more sampling is needed to predict order richness. From the observed trends we expect the Near Creek habitat type to show the highest total order richness and Juncus the lowest with this sampling mechanism.

4. DISCUSSION

Our study aimed to provide quantitative evidence of the effects of restoration on the habitat quality of these sites with the purpose of informing the NCCF on how successful their planting choices were when restoring these wetland systems. The results demonstrate that there is high variation in abundances and diversities of plant and invertebrate species across the restored wetlands. The 2007 and 2015 sites had the highest habitat quality, as they had higher average abundances and biodiversity when the means were significantly different (Table 1, 2). This does not support the hypothesis that older wetlands would have greater habitat quality, as the 2013 marsh had the lowest habitat despite being in the middle of the time series. Our results do not strongly support the hypothesis that vegetation types determine invertebrate community diversity. In the experimental design, strata were chosen based on the ecotone provided by the different types of vegetation. Strata with a greater number of plant species present corresponded with an increasing number of orders observed in both pan and sweep surveys, indicating the NC strata provided the greatest habitat quality (Figures 4, 5 and 6). This was further supported by the NC habitat having the greatest average abundance and diversity values in the majority of cases where the means were significantly different (Tables 3 and 4). However, there was no strong relationship between plant and invertebrate Shannon-Weiner biodiversity values (Figures 7 and 8).

Although we compared habitat quality between sites with the expectation that differences would be determined by age, variation in wetland design, such as where channels were dug out, could have affected the differences in habitat quality more significantly. The results from testing both hypotheses were inconclusive, but in future studies it may be useful to strategize how to sample wetlands of different designs. In the context of other chapters of this study, inundation,

denitrification, as well as a number of other factors could also influence the quality of habitats. Furthermore, it would be valuable to investigate where certain indicator species may occur. For example, these wetlands do provide habitat for some key species, i.e. “true bugs” of the order Hemiptera which have been shown to be a proxy for restoration success because of their response to disturbances (Orabi et al. 2010).

Some factors that may have confounded our findings in this study were wetland engineering, shoot size, and sampling time. Shoot size may have introduced error into the vegetation sampling due to differences in shoot size between species. Some species, such as J. romerianus tend to have thin shoots that can grow in high densities, whereas T. latifolia have broader shoots, meaning a similar vegetation area may be highly under or over represented based on shoot count. In the future, shoot diameter may be an important factor to measure. Sampling time may have introduced error because the rarefaction plots showed that species richness had not been completely sampled yet, so these relationships between diversity and vegetation may still be established in the future with more sampling effort.

This study implemented a quantitative assessment of habitat quality that offers many possible applications in other research. The methods established may be used to document and compare levels of diversity and abundance between other wetlands. This study also highlighted how sampling methods can have a significant effect on the type and diversity of samples collected. Additionally, this type of study design could be useful in the future for determining how to engineer wetlands to encourage certain cohorts of invertebrates or plants. Further sampling using the methodology described in this study, may clarify the relationship between wetland engineering and habitat quality. For the NCCF it would be valuable to compare habitat quality values to other areas within the North River Restoration project to determine which areas are progressing as planned. Also, many wetland restoration projects do not have qualitative goals (Kentula 2000), and studies such as this one can inform those who are considering restoration of base values of diversity and abundance of plants and insect populations as the restoration progresses. As demonstrated in this project, though very close geographically, community composition of each site varied dramatically. Going forward, wetland restoration projects should consider the goals of restoration and use these findings to plan the community they want to build.

5. CONCLUSION

We found that habitat quality did not increase with the age of the wetland. Rather than the habitat quality improving from the newest to the oldest wetland, 2007 and 2015 had greater habitat quality than 2013. There are some differences in invertebrate community composition between habitat types. The NC habitat appears to have the highest habitat quality of the strata types. Furthermore, a weak relationship was observed between vegetation diversity and invertebrate diversity in the 2007 and 2015 marsh. Our results suggest habitat quality is influenced by wetland design and may benefit from increased vegetation diversity.

SYNTHESIS

To assess the degree to which the North River wetlands preserve has achieved the goals of the North Carolina Coastal Federation, we quantified common metrics used for measuring ecosystem services. The metrics used included hydroperiod, carbon sequestration, denitrification, biodiversity, and water quality. In our initial hypotheses, we assumed that as a restored wetland ages, its ecosystem services would increase. However, we found restored wetland age to be insignificant for most metrics; of the various ecosystem services we examined, many were more dependent upon the design of the wetland.

We determined that the chronosequence and natural wetlands experienced varying degrees of inundation, though the 2013 wetland had a hydroperiod most similar to the natural wetland. Carbon burial rates were extremely high relative to the natural wetland, and the suspended sediment was rich in organic matter. We suspect the suspended sediment contributed to the high carbon burial rates. Farmland outfalls showed higher concentrations and loads of E. coli than restored wetland sites, suggesting that the wetland is effective at reducing fecal indicator bacteria. Denitrification rates increased with increasing inundation frequency, but showed no relationship with wetland age. Additionally, rates of denitrification across all sites were many times higher than the regional average for wetlands. Plant and invertebrate communities varied between habitat types, with the Near Creek habitat showing the greatest habitat quality.

In summary, of the three restored wetlands we examined, the wetland restored in 2007 had the highest carbon burial rate. The 2013 wetland had the highest denitrification rates, but the lowest carbon burial rates and habitat quality of the three restored wetlands measured. The 2015 wetland had the second-highest carbon burial rates and the lowest denitrification rates. These results indicate that designing a restored wetland that excels at all ecosystem services is not practical – there are tradeoffs associated with prioritizing a particular ecosystem service. Future restoration efforts should target desired ecosystem services and engineer the wetland to meet them accordingly. The results of this study will ideally aid in the design of restored wetlands by highlighting features that encourage different ecosystem services.

NUTRIENTS APPENDIX

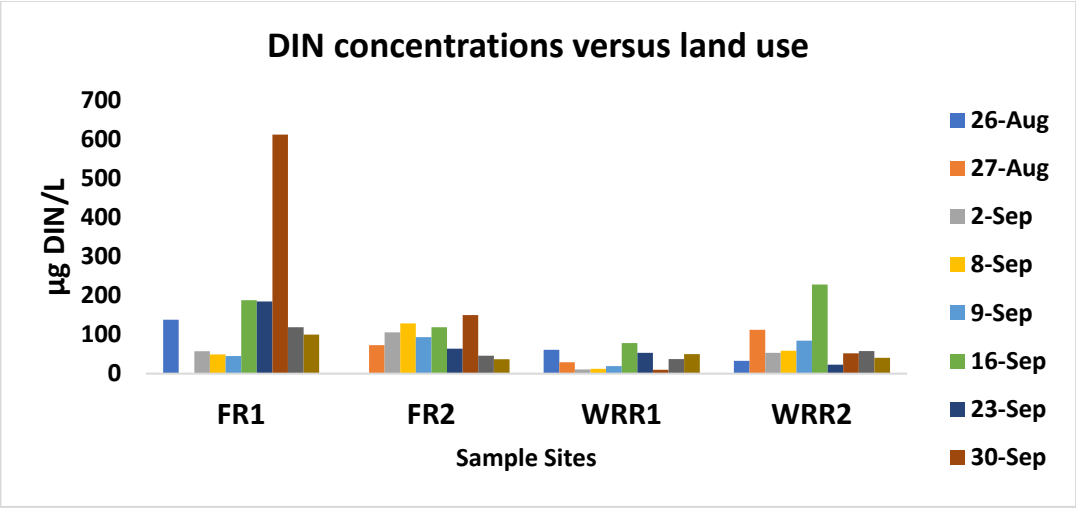

Figure 4. Variation of dissolved inorganic nitrogen (DIN) concentrations with land use and sampling date.

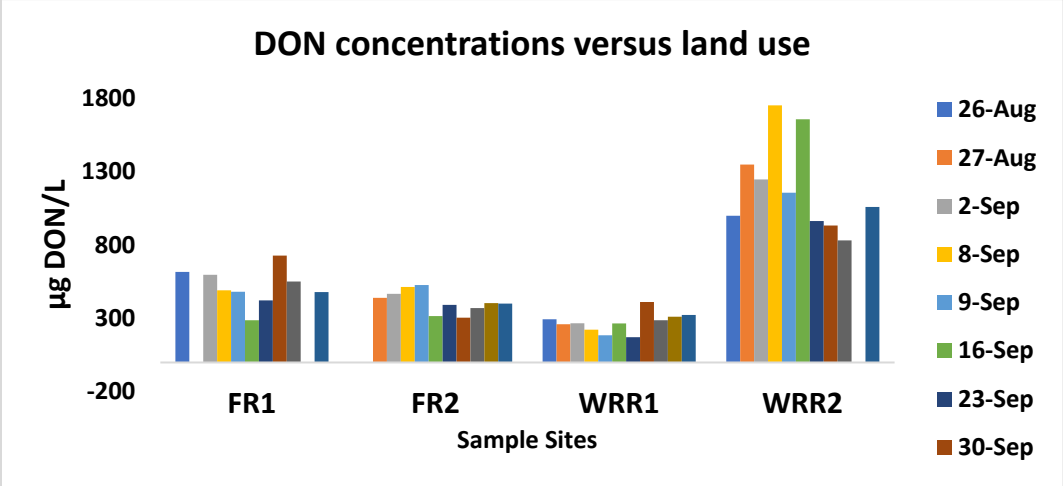

Figure 5. Variation of dissolved organic nitrogen (DON) concentrations with land use and sampling date.

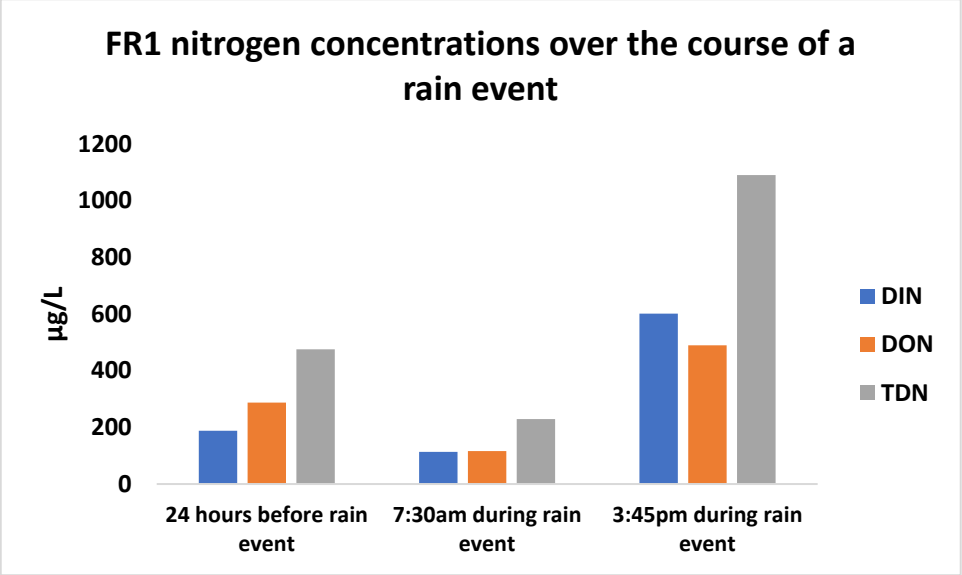

Figure 6. Variation in DIN, DON, and TDN concentrations over the course of a rain event on September 17th at FR1 outfall.

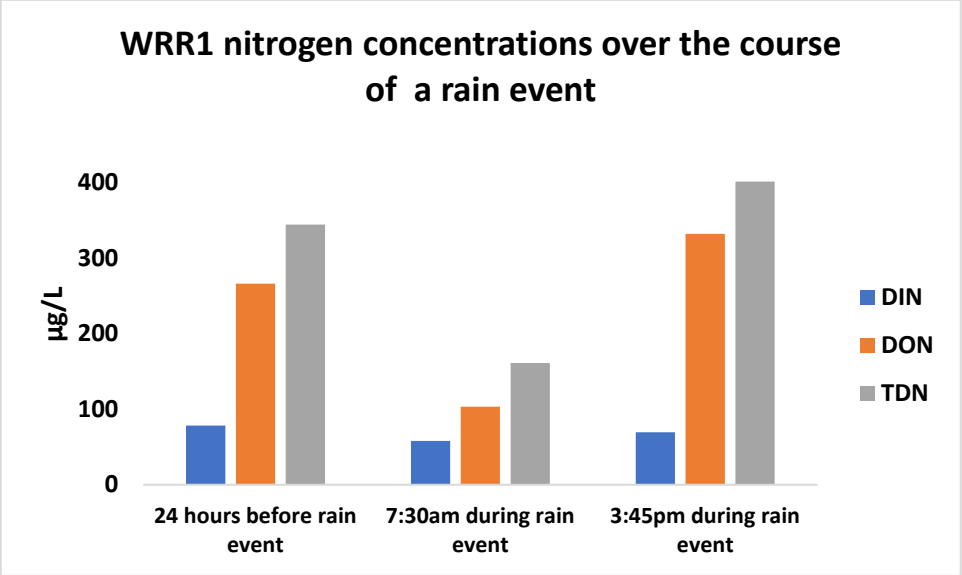

Figure 7. Variation in DIN, DON, and TDN concentrations over the course of a rain event on September 17th at WRR1 outfall.

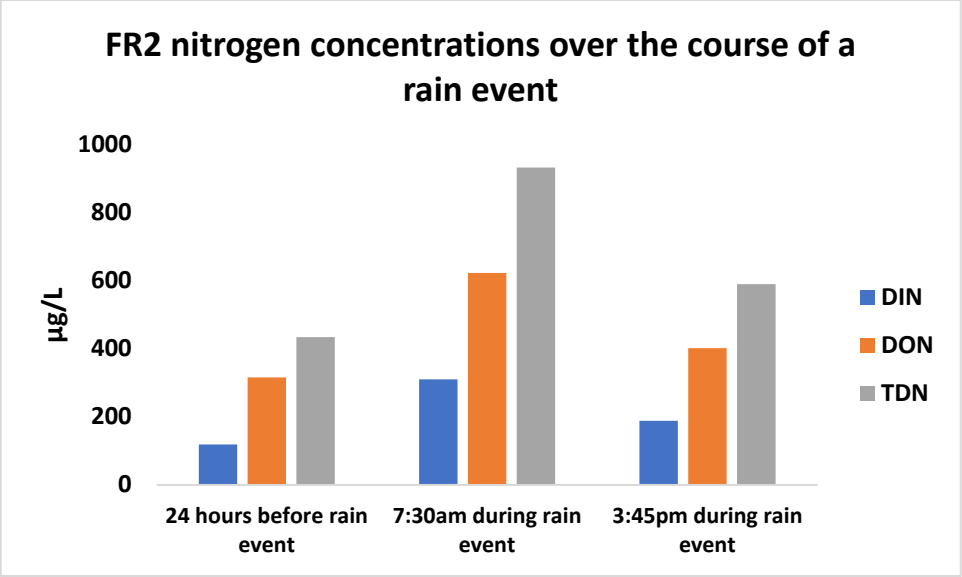

Figure 8. Variation in DIN, DON, and TDN concentrations over the course of a rain event on September 17th at FR2 outfall.

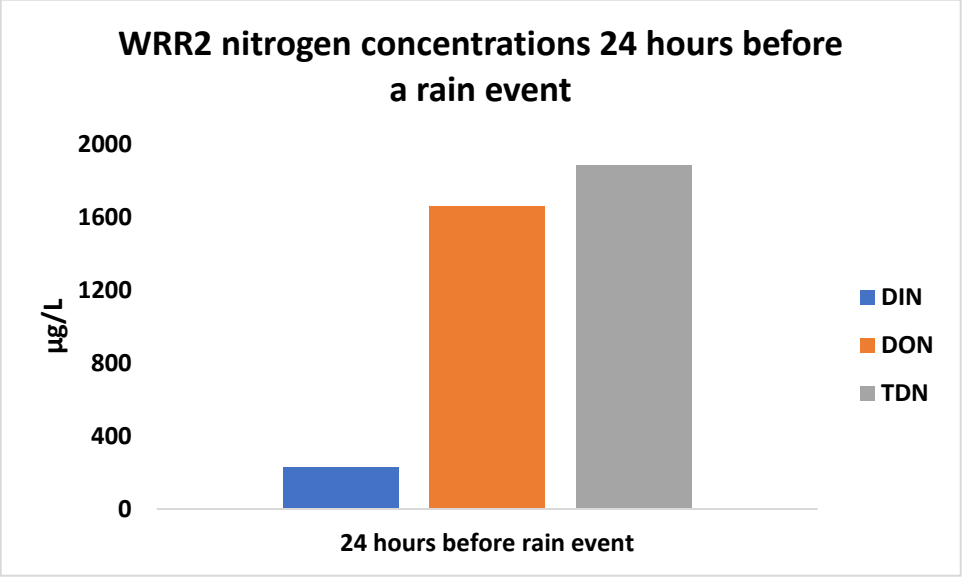

Figure 9. DIN, DON, and TDN concentrations the day before a rain event on September 17th at WRR2 outfall.

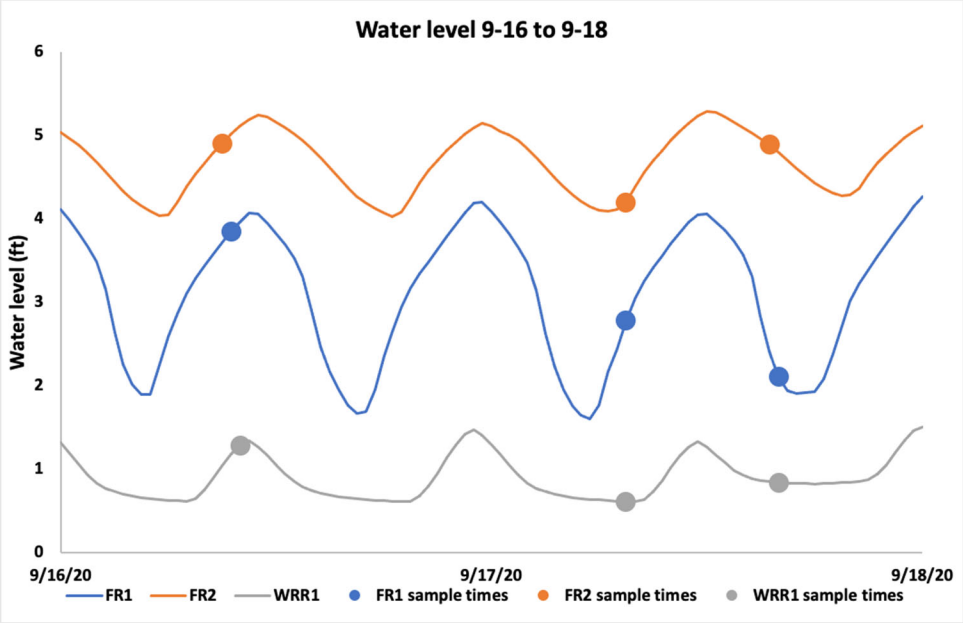

Figure 10. Water level and water sample collection times at FR1, FR2, and WRR1 outfalls from 9-16 to 9-18.

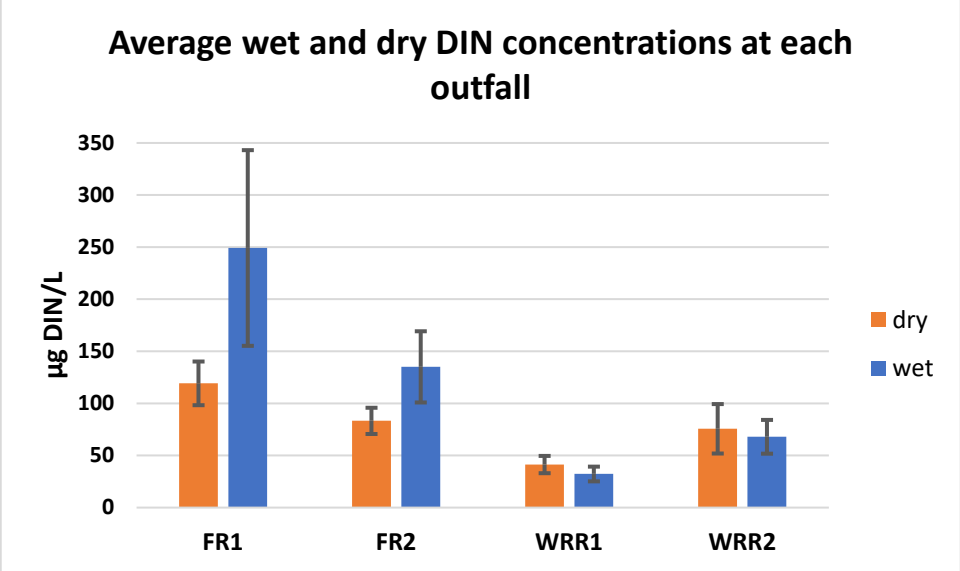

Figure 11. Average concentrations of DIN at each outfall on days with recorded rain events versus days without recorded rain events.

Table 1. Average flow in cubic feet per second at each outfall site

Outfall site	Average flow (ft^3/s)
Farm 1	3.90
Farm 2	9.89
Wetland 1	1.08
Wetland 2	0.0

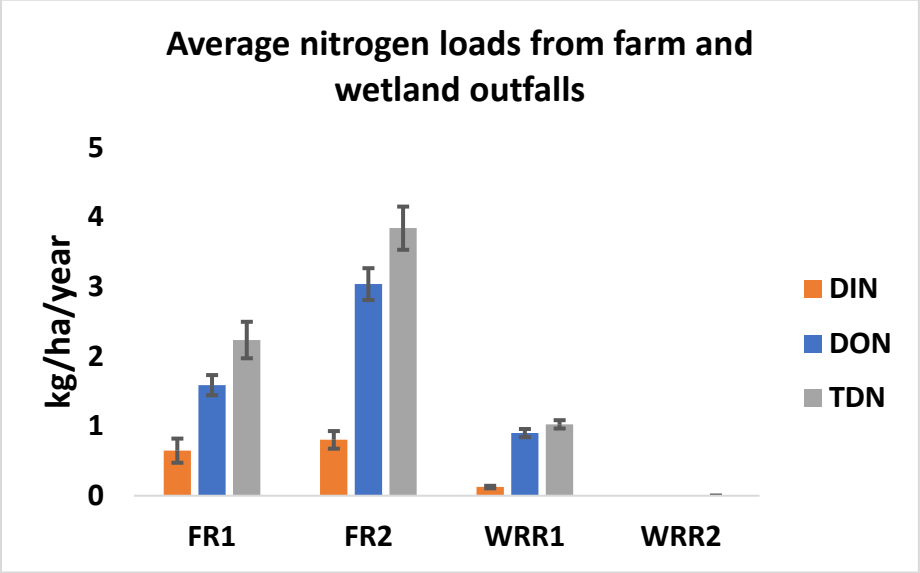

Figure 12. Average DIN, DON, and TDN loads from farm and wetland outfalls in kilograms per hectare per year.

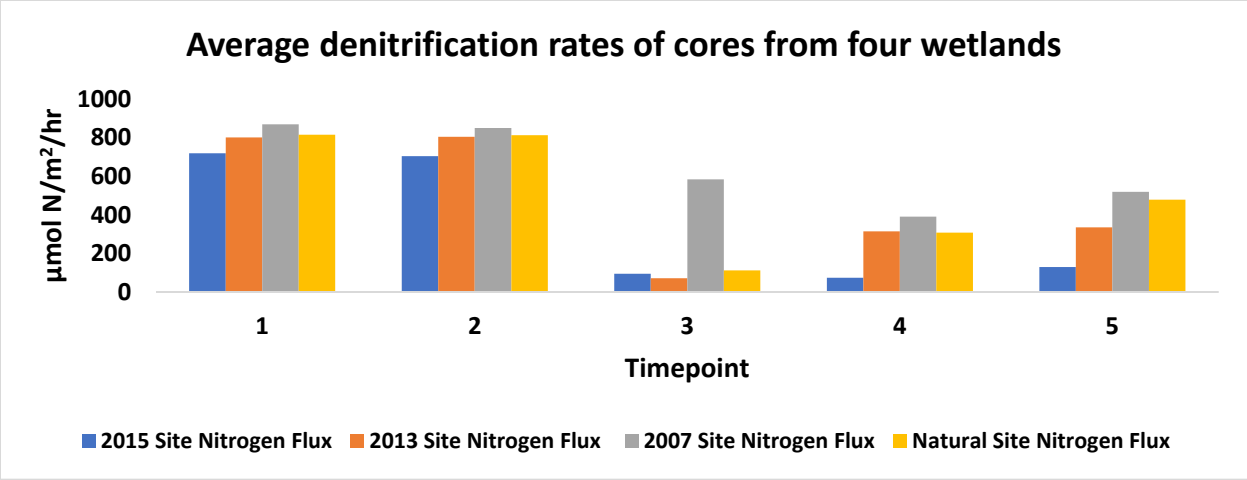

Figure 13. Denitrification rate of cores from each wetland by time point in the continuous flow denitrification experiment

Table 2. Nitrogen concentrations of core inflow water in micrograms per liter before and after addition of nitrate

	µg $N\text{-}NO_x/L$	µg $N\text{-}NH_4/L$	µg DON/L
Pre-addition	42.6	52.03	437.4
Post-addition	643	47.4	225.6

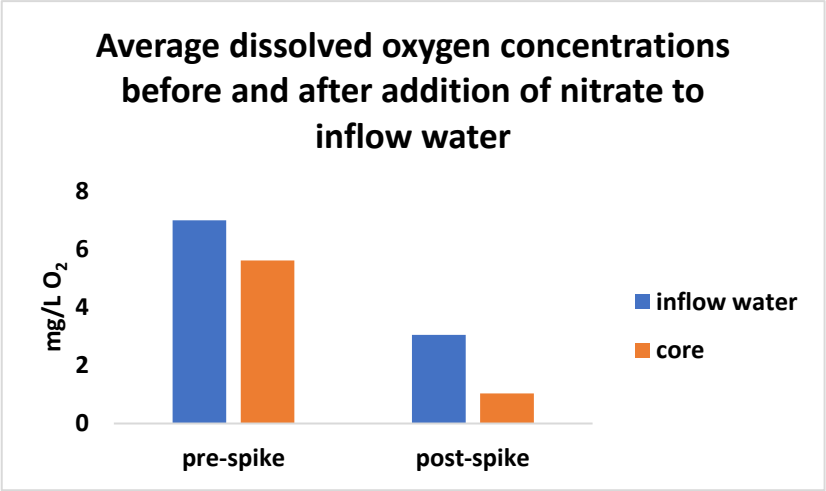

Figure 14. Average dissolved oxygen concentrations of inflow and outflow water in milligrams per liter from before and after addition of nitrate to inflow water.

Table 3. Average percent inundation of the locations of the cores for each wetland site

Wetland Site	% inundation
2007	39
2013	74
2015	25
Natural	67
Regional Average	50

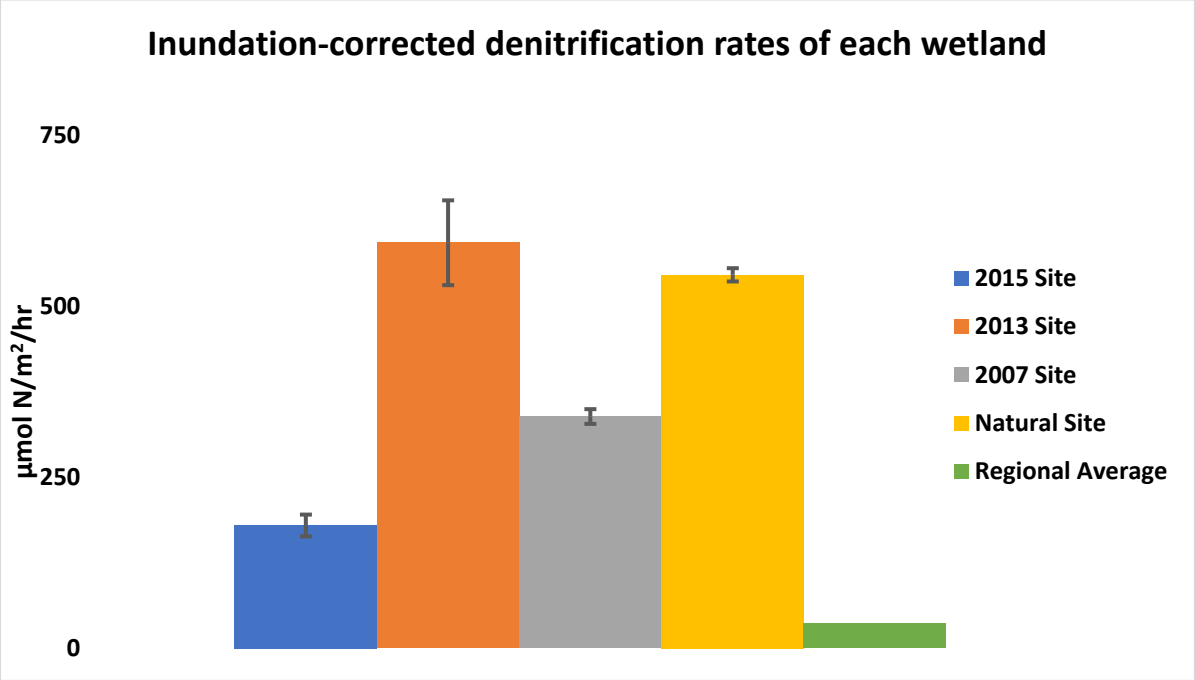

Figure 15. Comparison of denitrification rates across wetland sites after correcting for inundation

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